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IMMUNOLOGY OF ACUTE RADIATION INJURY

by R. V. Petrov

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IMMUNOLOGY OF ACUTE RADIATION INJURY

[Following is a complete translation of a Russian-language book by R. V. Petrov entitled Immunologiya Ostrogo Luchevogo Porazheniya (English version above), Gosatomizdat, Moscow, 1962, pages 1-267.]

Table of Contents

| | |
|------------------------|---|
| Introduction | 1 |
|------------------------|---|

~~First Part~~

← The Effect of Radiation on Antimicrobial Immunity

Chapter I. Sensitivity to Infection after Irradiation.

| | |
|---|----|
| 1. Infection with Pathogenic Microorganisms | 3 |
| 2. Infection with Conditionally Pathogenic Microorganisms | 15 |
| 3. Time of Increase in Sensitivity to Infection | 17 |
| 4. Bibliography | 24 |

Chapter II. Basic Immunity Mechanisms

| | |
|--|----|
| 1. The Normal Microflora | 31 |
| 2. Tissue Permeability and the Segregating Function of the Reticulo-Endothelial System | 40 |
| 3. Bactericidal Properties of the Tissues | 54 |
| 4. Phagocytosis | 58 |
| 5. Antibody Formation | 65 |
| 6. Species Resistance | 75 |
| 7. Bibliography | 78 |

Second Part

← Infectious Processes in the Irradiated Organism

Chapter III. Endogenous Infection

| | |
|--|-----|
| 1. Sources of Endogenous Infection | 89 |
| 2. Times of Occurrence of Bacterial Invasion | 92 |
| 3. The Role of Endogenous Infection in the Pathogenesis of Radiation Sickness | 96 |
| 4. Principles of Antibiotic Therapy in Acute Radiation Sickness | 101 |
| 5. Bibliography | 114 |
| Chapter IV. Exogenous Infections | |
| 1. Characteristics of the Course of Infections . . | 120 |
| 2. Activation of Latent Infection | 130 |
| 3. Effectiveness of Specific Prophylaxis | 131 |
| 4. Various Experimental Examples Illustrating the Characteristics of Infectious Processes . . | 138 |
| 5. Bibliography | 161 |

Third Part

Noninfectious Immunology of Radiation Sickness

| | |
|------------------------|-----|
| Introduction | 170 |
| Bibliography | 173 |

Chapter V. Antigenic Properties of Tissues of Irradiated Animals

| | |
|---|-----|
| 1. Change in the Antigenic Properties of Tissues after Irradiation | 175 |
| 2. Species, Organ and Organoid Specificity of Tissue Antigens | 185 |
| 3. Circulation of Tissue Antigens in the Blood Stream | 196 |
| 4. Reasons for the Change in the Antigenic Properties of Tissues | 200 |
| 5. The Specificity of Change in the Tissue Proteins. | 213 |
| 6. Bibliography | 219 |

Chapter VI. C-Reactive Protein and Radiation Sickness

| | |
|---|-----|
| 1. The Nature of C-Reactive Protein | 225 |
| 2. The Appearance of C-Reactive Protein in the Blood of Patients During Radiation Therapy. . | 229 |

| | | |
|---|---|-----|
| 3. | C-Reactive Protein in the Blood of Monkeys with Acute Radiation Sickness | 236 |
| 4. | Bibliography | 245 |
| Chapter VII. <u>Reactions of the Body to Tissue Antigens</u> | | |
| 1. | Toxicity of Tissues of Irradiated Animals. | 249 |
| 2. | Is Autoimmunization of an Irradiated Organism Possible? | 257 |
| 3. | Autoantibodies in the Blood of Irradiated Animals. | 262 |
| 4. | Demonstration of the State of Sensitization to Tissue Antigens | 272 |
| 5. | Bibliography | 281 |
| Chapter VIII. <u>Study of the Effect of Immune Sera Against Tissues of Irradiated Animals</u> | | |
| 1. | Aggravating Effect of Whole Sera | 288 |
| 2. | The Favorable Effect of Adsorbed Sera | 295 |
| 3. | Bibliography | 312 |

Conclusion

| | | |
|----|--|-----|
| 1. | Antimicrobial Immunity in Radiation Injury. | 315 |
| 2. | Infectious Processes in Radiation Injury. | 320 |
| 3. | Noninfectious Immunology of Radiation Injury | 323 |

Introduction

In recent years, in connection with the precipitous development of nuclear physics and the utilization of radioactive emanations in science, new branches of medicine and biology have been developed: radiation genetics, radiation biochemistry, radiation microbiology, and others. A new branch of knowledge has also been born -- radiation immunology -- part of which, studying radiation sickness, can be called "the immunology of radiation sickness."

In the Soviet and foreign literature the following, not entirely complete definition of this new branch of science has been given. It is customarily considered that radioimmunology includes the study of the effect of radiation on anti-infectious immunity and on infections. Several years ago, V. L. Troitskiy wrote the following: "Included in the tasks of radioimmunology is the study of the effect of ionizing radiation on infection and immunity as well as methods of controlling infectious complications of radiation sickness and the immunity disorders in it."

At the present time, this formulation no longer exhausts the subject. Many facts have been accumulated attesting to the idea that immunological problems in radiation sickness are not limited to subjects associated with infectious-disease immunology. Every year, progressively more data are appearing which prove the existence of problems of radiation sickness of current importance lying in the sphere of non-infectious immunology. These are works on the change in antigenic properties of tissues after irradiation, the role of tissue antigens in the pathogenesis of radiation sickness, the possibility of acting immunologically on them. Of exceptional interest and practical importance are works on the study of C-reactive protein in radiation injury. Problems arising in connection with transplantation of tissues to irradiated organisms are very closely allied to non-infectious immunology of radiation sickness.

The basic task of this book is a substantiation and characterization of the immunology of radiation sickness as a whole as a subject consisting of three main divisions:

1. The effect of radiation on antimicrobial immunity.
2. Infectious complications in irradiated animals.
3. Non-infectious immunology of radiation sickness.

Thereby, it was necessary not only to throw light as completely as possible on the literature of recent years but also to describe in

detail the results of our own studies. The relative degrees of importance of our own experimental data for substantiating and characterizing various divisions are different.

The first division of immunology of radiation sickness at the present time has been studied very completely. Our own studies illustrate and enrich only some of its aspects with new data: antibody-formation during the infectious process, the characteristics of passive immunity to anaerobic infection, the rate of elimination of pathogens from the irradiated organism and changes in the normal microflora.

The second division is also extensively represented in the literature. Our own data permit us to distinguish certain phases in the development of autoinfections in radiation sickness, to clarify the principles of antibiotic therapy of it, to demonstrate the characteristics of courses of a number of infections, and to establish the effectiveness of prophylaxis and treatment of them.

The third division is the newest branch of immunology of radiation sickness, and our own data play the greatest part in substantiating and characterizing it.

In making some studies the work required the qualifications of other specialists -- a number of experiments was performed in combination with microbiologists, physiologists, biophysicists, a biochemist, hematologist, pathologist, and clinician. In connection with this, I should like to take advantage of this occasion and I consider it my pleasant duty to express my appreciation to V. N. Benevolenskiy, Ye. K. Dzhikidze, A. L. Zhuravlev, L. I. Il'ina, N. N. Klemparskaya, M. A. Lagun, G. M. L'vitsyna, N. L. Melik-Pashayeva, A. S. Petrov, V. D. Rogozkin, M. F. Sbitneva, A. B. Tsypin and V. V. Shikhodyrov for the work which they put into our combined studies.

FIRST PART

THE EFFECT OF RADIATION ON ANTIMICROBIAL IMMUNITY

Chapter I

Sensitivity to Infection after Irradiation

1. Infection with Pathogenic Microorganisms

Beginning with the 1890's the literature has been supplemented with progressively newer data, which illustrates the change in the sensitivity of irradiated animals to infection with pathogenic microorganisms. Depending on the dose of irradiation used, investigators have recorded an increase or decrease in sensitivity. Thereby, in the first few years, in connection with imperfect methods of dosimetry of ionizing radiation, controversial conclusions were expressed concerning the effect of this type of energy on anti-infectious immunity. However, in subsequent years it was established that the so-called stimulatory effect is exerted only by small doses of radiation, while favorable effects can be obtained only for certain infections with local irradiation (inflammatory processes, gas gangrene and others). W. Taliaferro and L. Taliaferro (1951) have given a bibliography for works of the early period. Bibliographic data for recent years have been presented most fully in I. A. Pigalev's report (1955) to the International Conference in Geneva, P. N. Kisilev's report (1956) to the Thirteenth All-Union Congress of Microbiologists, in R. V. Petrov's review (1958), in the monographs of N. N. Klemparskaya, O. G. Alekseyeva, R. V. Petrov and V. F. Sosova (1958) and those of V. L. Troitskiy and M. A. Tumanyan (1958).

For more than 50 years of experimentation in the field of infectious immunology of radiation sickness investigators tested the sensitivities of irradiated animals to various infectious-disease pathogens. Among them, mention may be made of the pathogens of tuberculosis (Morton, 1916; L. B. Beylin and others, 1956), malaria (Taliaferro, 1945; Singer, 1953), trypanosomal infection (Naiman, 1944), pneumococci (Corper, Chovey, 1920), streptococci (Schechmeister, Adler, 1945, 1953), the Breslau bacillus [*Salmonella breslau*] (Gowen, Zell, 1945; V. N. Sivertseva, 1956) and others.

The works of recent years only expanded the scope of our

knowledge and elucidated the details of interaction of the irradiated organism and bacteria. The fundamental regularity -- marked increase in the sensitivity of irradiated animals to pathogens of infectious diseases -- has been known for many years now. As an illustration we shall dwell on some of the most interesting examples in this respect.

A high degree of susceptibility of irradiated animals to oral, that is, natural, infection with pathogens of intestinal diseases through the example of paratyphoid was demonstrated by L. A. Yakovleva and coauthors (1957) and Korner (1958). L. A. Yakovleva and coauthors experimented on monkeys; Korner, on mice.

Fourteen macaques were infected orally with a strain of paratyphoid B in a dose of from $30 \cdot 10^9$ to $50 \cdot 10^9$ microbes depending on the animal's body weight. Of 14 monkeys only one died of paratyphoid fever. If the monkeys were infected after irradiation with x-rays in a dose of 300 r the results were different. Of five monkeys which received the same dose of microbes four died of paratyphoid fever five days after irradiation. The disease lasted a total of five-six days. In itself, irradiation with a dose of 300 r does not cause death of monkeys; it brings about the development of radiation sickness, which occurs for a short time and in a mild form.

Among the numerous works on the infection of irradiated animals with the pathogens of different infectious diseases those in which natural infection conditions were created are certainly of special interest. Bond and coauthors in 1952 made a study of an endemic pulmonary infectious disease of undetermined etiology in rats in one of the nurseries. Injury to the lungs consisted of the occurrence of areas of consolidation which microscopically were of a proliferative nature. The rate of infection in the pack of rats reached 40 percent. When a new strain of rats free of this infection was mated with infected animals, there were no cases of infection for the 25 days of observation. At the same time, among the irradiated rats 17 percent were infected and showed typical pulmonary manifestations of the disease. Schechmeister and Adler (1953) showed an increase in the sensitivity of irradiated animals to natural infection occurring after the mating of animals infected with tuberculosis with non-infected animals.

The following question is perfectly in order: how many times more sensitive are irradiated animals than normal and infected animals? The answer to this is given by a number of quantitative studies, in which the LD₅₀ for irradiated and non-irradiated animals was determined.

V. L. Troitskiy and M. A. Tumanyan (1958) determined the indices of resistance after intraperitoneal infection of irradiated mice with the pathogens of typhoid fever and dysentery. The index of resistance was defined as the ratio of the LD₅₀ for irradiated animals to the LD₅₀ of non-irradiated animals. It was established that these indices were equal to 0.2-0.3 after x-ray irradiation of mice with a sublethal dose (400 r).

After aerogenic infection of mice irradiated with a dose of 350 r with the use of hemolytic streptococci (Schechmeister and others, 1952), the maximum reduction of the LD₅₀ for irradiated mice was also by five times. In experiments with intraperitoneal infection with *S. enteritidis* (Schechmeister and others, 1952, 1953) the infecting dose for irradiated mice was hundreds of times less.

V. L. Troitskiy and M. A. Tumanyan (1958) describe a certain degree of resistance of monkeys to artificial infection of them with dysentery pathogens. Oral administration of $100 \cdot 10^9$ microbes to healthy monkeys does not lead to a case of dysentery. In irradiated animals this infectious disease develops even after the administration of $10 \cdot 10^9$ - $20 \cdot 10^9$ microbes.

We should dwell separately on experiments of infecting irradiated animals with pathogenic viruses. As is well known, for reproduction of a virus it must be included in the cell metabolism (K. S. Sukhov, 1950; L. A. Zil'ber, 1956), which is markedly disturbed after irradiation (Abrams, 1951; A. M. Kuzin and Ye. V. Budilova, 1953; L. L. Il'ina, 1957). Is it not possible that a change in metabolism can delay virus reproduction and, by the same token, reduce the susceptibility of the irradiated organism to virus infections? This question requires careful analysis. A number of studies give a negative answer to this. In 1935, Brodie and others reported that intraperitoneal and intracerebral infection of irradiated white mice with the pathogen of poliomyelitis reveals that their resistance to this virus is reduced. Syverton and others (1952) showed that irradiation of white mice with a dose of 200-400 r increases their sensitivity to this virus by four times.

De Gara and Furth (1945), Bentler (1952), A. A. Smorodintsev (1955, 1957), V. N. Sivertseva (1955), O. P. Peterson and L. A. Kozlova (1956, 1957) and others showed a reduction of the resistance of irradiated white mice and rats to the influenza virus. Ye. A. Brodskaya and O. G. Petrovskaya (1960) described increased sensitivity of irradiated mice to the Cocksackie virus. Reduction of resistance to viruses of lymphocytic choriomeningitis, encephalomyelitis,

tick-borne encephalitis, influenza and rabies after irradiation was shown by P. I. Remezov (1960).

O. P. Peterson and I. A. Kozlova in 1957 reported that they were able to reduce the natural resistance not only in mice or rats to the influenza virus but also in guinea pigs. For this, it was necessary to irradiate the guinea pigs with doses of 200-400 r. After the infection the guinea pigs develop a clinically expressed disease with the development of the corresponding pathological process in the lungs. At the same time, some authors report an increase in the resistance of irradiated animals to a number of viruses, for example, of mice to the swine influenza virus (Dubin and others, 1946) and to the street virus of rabies (Ye. I. Sklyanskaya, 1957). Ye. I. Sklyanskaya explains this phenomenon specifically by a reduction in the power of reproduction of the virus in the body cells, as a result of a change of metabolic processes under the influence of irradiation. However, at the present time there is no basis for such a conclusion. This is evidenced, first of all, by the sparsity of data concerning increase in the resistance of totally irradiated animals to infection with viruses. Secondly, there are materials in existence which indicate that even in lethally irradiated cells viruses continue to multiply until the cell dies, whereby more viruses accumulate there than in intact cells. We have in mind the works of Labaw (1953), Blumenthal and others (1953), Schneider and Cheever (1954), Weiss and Dressler (1958).

The first author irradiated the colon bacillus with doses of 160,000-735,000 r and produced a burst size of T6+ bacteriophage in these surviving cells which was tens of thousands of times greater. Blumenthal and coauthors irradiated chick embryos with doses of 250-500 r and observed an acceleration of virus reproduction. Schneider and Cheever, Weiss and Dressler did not observe an inhibition of virus reproduction in irradiated tissue cultures.

The accumulations of greater than normal quantities of viruses in tissues of irradiated animals are reported by scores of authors (A. A. Smorodintsev, 1957; V. N. Sivertseva, 1956; I. A. Kozlova, 1958; Ye. A. Brodskaya and O. G. Petrovskaya, 1960). Finally, the experiments of Ye. I. Sklyanskaya may be evaluated differently. Ye. I. Sklyanskaya observed an increase in resistance or an absence of reduction of resistance only with respect to the neurotropic viruses, but not the encephalomyocarditis virus (MM) or yellow fever virus (the 17 D strain with a low degree of neurotropism). However, as is well known, morphologic and biochemical changes in nerve tissue after irradiation are observed to a considerably lesser degree than in other

tissues (I. I. Ivanov and others, 1956; N. A. Krayevskiy, 1957). In this respect, nerve cells are radioresistant. Conversely, the functions of the nervous system are primarily impaired after irradiation (A. V. Lebedinskiy, 1955; M. N. Livanov, 1956). Therefore, it seems to us that the lower degree of expression of some, particularly neurovirus infections, in irradiated animals may be the result of lack of manifestation of a number of symptoms as the result of the predominance of inhibitory processes in the central nervous system in radiation sickness. Such an inhibitory effect of the central nervous system on infectious processes has been studied in detail by P. F. Zdrodovskiy (1950). In any case, these exceptions are isolated ones and we can speak of the general rule of increase in the sensitivity of irradiated animals to viruses.

The sensitivity to infection with rickettsias is also increased in radiation sickness (Zinsser, Castaneda, 1932; Liu and others, 1946). There are experimental and clinical observations attesting to an increase in the sensitivity of the irradiated organism to fungi also (Syvertson and others, 1952; Ye. A. Karpovich, 1957; A. Ya. Prokopychuk and N. A. Kostenich, 1957).

Our own experiments on the study of the sensitivities of irradiated animals to infection were made with the pathogens of gas gangrene, tetanus and leptospirosis (R. V. Petrov, 1957). Experiments with the pathogen of gas gangrene were performed on white mice weighing 25-28 grams and on guinea pigs weighing 500-700 grams.

The irradiation was carried out with gamma-rays on a cobalt gamma-active source 35 centimeters long. The dose rate at a distance of 50 centimeters was equal to 20.4 r per minute. For the infection 24-hour cultures of *B. perfringens* No 243 and *B. tetani* No 280 grown out on Kitt-Tarozzi medium were used. For the purpose of finding out the degree of sensitivity of irradiated animals to the pathogen of gas gangrene a dose of the microbe was titrated out which led to the obligatory development of infection and death in 10-20 percent of the mice. This dose was 0.2 cc of a mixture of a 1:1 culture of the pathogen with 10 percent calcium chloride solution added as a tissue-necrotizing agent. The infection was introduced into the thigh muscle. The gas gangrene infection developed as early as after five-six hours; death occurred after two-three days.

Six hundred animals were irradiated with a dose of 439 r, which was the minimum lethal dose and caused the death of three-four percent of the mice from radiation sickness. The experimental animals were infected in batches of 50, beginning with the first day of

irradiation, every two, four, etc. days for 20 days. Simultaneously, a group of control mice was infected. The time of death of the irradiated animals depended on the time elapsing between irradiation and infection.

Below, we shall specially discuss the time of increase in the sensitivity to infection after the effect of ionizing radiation. Here, we are directing attention only to the fact of the greater mortality of irradiated mice from gas gangrene (Fig 1). For example, in the case of infection on the fifth day the mortality of irradiated mice from gas gangrene infection amounted to 70 percent instead of the 10-20 percent in the control.

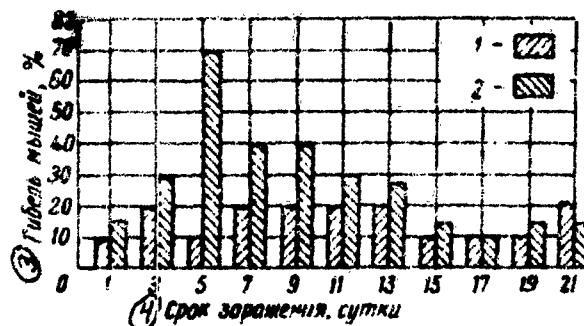


Fig 1. Change in the Sensitivities of Irradiated White Mice to Infection with the Gas Gangrene Pathogen at Different Periods after Irradiation: 1. Control; 2. Irradiated; 3. Mortality rate of mice, %; 4. Time of infection, days.

In experiments on guinea pigs the gas gangrene pathogen was introduced into the gap of an incised skin-muscle wound one centimeter in length. The dose of the pathogen amounted to 0.1 cc of a 24-hour culture. The wound was inflicted 10 hours after irradiation of the guinea pigs with a dose of 367 r. The culture was introduced into the wound immediately after it was inflicted. With this manner of introducing the infection the infectious disease has a more prolonged course than after intramuscular infection and causes the death of 45 percent of the animals in two weeks.

The experiment was represented by three groups of animals: 20 guinea pigs infected after irradiation, 20 which were only infected, and 15, which were only irradiated. On Fig 2 the time of death of the guinea pigs is shown. The animals which were simply irradiated began to die only on the eighth day, whereas the majority of those infected after irradiation died with signs of a striking gas gangrene infection. In the control group the gas gangrene infection was expressed to a much lesser degree.

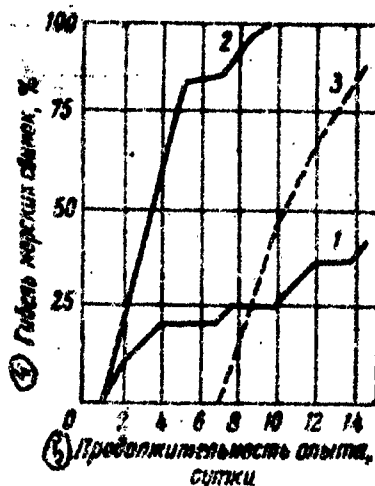


Fig 2. Time of Death of the Irradiated and Control Guinea Pigs Infected with Gas Gangrene Pathogen: 1. Infection control; 2. Irradiation and infection; 3. Irradiation control; 4. Mortality rate of guinea pigs, %; 5. Duration of experiment, days.

The study of the sensitivities of irradiated animals to infection with the tetanus pathogen was made by us in cooperation with M. A. Lagun on white mice irradiated with a dose of 367 r. Fifty irradiated and 50 control animals were infected. For this, a 24-hour culture of the microbe was diluted 50 times with physiological saline solution; the suspension obtained was mixed with 10 percent calcium chloride solution in a ratio of 1:1 and injected intramuscularly in a dose of 0.1 cc. This method of infecting mice led to the development of a

typical picture of ascending tetanus in them with 100 percent mortality of the animals within six days. The irradiated animals infected 10 hours after irradiation showed increased sensitivity to tetanus -- all the mice died within four days after the infection (Fig 3). All the control mice survived.



Fig 3. Time of Death of Irradiated and Control White Mice Infected with Tetanus Pathogen: 1. Infection control; 2. Irradiation and infection; 3. Mortality rate of mice, %; 4. Duration of experiment, days.

The experiments with leptospirosis were performed on rabbits weighing 1.5-three kilograms, guinea pigs weighing 150-200 grams, white mice weighing 10-15 grams and bunnies weighing 250-260 grams. The animals were subjected to a whole body irradiation with sublethal doses of x-rays. Rabbits were irradiated on a three-tube apparatus with a dose of 500-600 r, 120 kv, 20 ma with a filter (0.5 mm Cu + 1 mm Al) at a distance of 60 centimeters from the source, and a dose rate of 30 r per minute. White mice (350-400 r), guinea pigs (200 r) and bunnies (200 r) were irradiated with 180 kv, 15 ma using the same filter. The distance from the source was equal to 50-40 centimeters; the dose rate, 30-37 r per minute. For the infection two strains of leptospiras were used: *L. icterohemorrhagiae* and "Krysa Ramenka." In its serological properties the first strain was identical with the

pathogen of Weil's disease, being pathogenic for calves, colts, puppies, guinea pigs and bunnies. The second strain was very similar to *L. canicola* in its antigenic properties, being pathogenic for young guinea pigs.

The leptospiras were cultivated at 25° C on doubly distilled water with five percent rabbit serum. In the experiment cultures were used which contained 80-100 leptospiras per microscope field. The rabbits were infected intravenously with the "Krysa" strain in a dose of 1.5-two cc. Rabbits and guinea pigs were infected intraperitoneally with a culture of the *L. icterohemorrhagiae* pathogens, which were injected into the guinea pigs in a quantity of 0.05-0.5 cc and into the rabbits in a quantity of 0.2 cc. White mice were infected with both strains of leptospiras (0.2 cc intraperitoneally).

The infection of bunnies and guinea pigs gave rise to the development of a clinically expressed infectious disease. In rabbits and white mice leptospirosis had a latent course, and the non-irradiated animals did not die. After infection with leptospiras not a single case of death of normal rabbits or white mice was observed. At the same time, animals infected after irradiation died in large numbers or entirely, despite the fact that the doses of irradiation were sublethal. In the experiments, 190 white mice and 27 rabbits were used.

In Table 1 the results of infection of the rabbits with the "Krysa" leptospira culture are shown. The infected animals did not die without irradiation. Of five rabbits which were simply irradiated with a dose of 600 r one died, and of six rabbits infected after irradiation five died. All four rabbits infected 24 hours after irradiation with a dose of 500 r also died. Of the four rabbits which were simply irradiated three remained alive.

From Table 2, in which the results of infection of irradiated and non-irradiated white mice are shown, it is seen that infection with a leptospiral "Krysa" culture in animals irradiated with a dose of 350 r ended in the death of 22 percent of them, whereas after irradiation alone only 10 percent died. After infection with the same leptospiras mice irradiated with 400 r died to the extent of 85 percent where there was a 40 percent mortality in those simply irradiated. The results of the experiment with infection of mice with *L. icterohemorrhagiae* are even clearer. The presence of clinical signs of leptospirosis (jaundice) as well as the constant finding of leptospiras not only in the kidneys but also in the blood of the bodies is evidence that the animals died with well-developed leptospiral infection.

The next series of experiments was performed on irradiated

Table 1

Results of Infection of Rabbits with the Leptospirosis Pathogen

| ① Содержание опыта | ② Число животных | ③ Доза облучения, р | ④ Срок между облучением и заражением, ч | ⑤ Количество погибших животных |
|----------------------------|---------------------|------------------------|--|-----------------------------------|
| ⑥ Контроль заражения | 8 | — | — | 0 |
| ⑦ Облучение и заражение | 4 4 2 | 600 500 600 | 2—3 24 48 | 3 4 2 |
| ⑧ Контроль облучения | 5 4 | 600 500 | — — | 1 1 |

1. Content of experiment; 2. No. of animals; 3. Dose of radiation, r; 4. Time between irradiation and infection, hours; 5. No. of animals which died; 6. Infection control; 7. Irradiation and infection; 8. Irradiation control.

bunnies and guinea pigs. In the first experiment, performed on guinea pigs (30), a culture of *L. icterohemorrhagiae* which had been passaged for a long time through synthetic nutrient media, as a result of which the virulence of the leptospiras had been reduced, was utilized for the infection. The result of this was a low late mortality of animals which were simply infected. From Table 3 it is seen that of 10 guinea pigs (Nos 31-40) infected with the pathogen of leptospirosis, only three died on the 19th-60th day after infection. On cultures of kidney emulsions leptospiras were found. Two guinea pigs (No 33 and No 34) were sacrificed on the ninth and 10th days after infection. In the kidneys leptospiras were found. Of 10 guinea pigs which were simply irradiated, four died on the 10th-15th day after irradiation. When irradiated guinea pigs (Nos 21-30) were infected, they all died on the ninth-

Table 2

Death of White Mice Infected with Leptospirosis Pathogens

| 1 Содержание опыта | 2 Количество мышей в опыте | 3 Доза облучения, r | 4 Заражение | | 7 Количество павших мышей | 8 Время гибели после заражения, сутки | 9 Желтуха |
|-----------------------------|-------------------------------|------------------------|-----------------------------|------------------------|------------------------------|--|--------------|
| | | | 5 после облучения, сутки | 6 штаммы лептоспир | | | |
| 10 Контроль заражения | 40 | — | — | 13 «Крыса» | 0 | — | — |
| | 20 | — | — | Иктеро-геморрагические | 0 | — | — |
| | 10 | — | — | 14 | 0 | — | — |
| 11 Облучение и заражение | 40 | 350 | 15 В день облучения | 13 «Крыса» | 9 | 4—30-е | — |
| | 20 | 400 | 4-е | Иктеро-геморрагические | 17 | 3—24-е | — |
| | 10 | 400 | 40-е | 14 | 10 | 6—30-е | + |
| | 15 | 400 | 72-е | Иктеро-геморрагические | 10 | 15—30-е | + |
| | 16 | 350 | — | — | 14 | 6—30-е | + |
| 12 Контроль облучения | 20 | 350 | — | — | 2 | 6—11-е | — |
| | 30 | 400 | — | — | 13 | 3—29-е | — |

1. Content of experiment; 2. No. of mice in the experiment; 3. Dose of radiation, r; 4. Infection; 5. After irradiation, days; 6. Strain of leptospira; 7. No. of mice which died; 8. Time of death after infection, days; 9. Jaundice; 10. Infection control; 11. Irradiation and infection; 12. Irradiation control; 13. "Krysa"; 14. L. icterohemorrhagiae; 15. On day of irradiation.

14th day with signs of marked leptospirosis (jaundice, profuse hemorrhages into the muscles). Leptospiras were found not only in the kidneys but also in the liver and in the blood.

Similar results were obtained in experiments on bunnies. Of three infected animals two died on the seventh day; of three irradiated

Table 3

Results of Infection of Guinea Pigs with a Culture of
L. Icterohemorrhagiae

| ① Содержание опыта | ② № самки | ③ Вес самки, г | ④ Доза об- лучения, р | ⑤ Доза за- ражения (в дозу облуче- ния), мл | ⑥ Время гибели, сутки | ⑦ Желтуха |
|-------------------------------|-----------------|----------------------|--------------------------------|---|-----------------------------|--------------|
| ⑧ Заражение (контроль) | 31 | 180 | — | 0,05 | Выжила ⑪ | + |
| | 32 | 145 | — | 0,05 | Забита на 10-е сутки ⑫ | |
| | 33 | 151 | — | 0,1 | Выжила ⑪ | — |
| | 34 | 160 | — | 0,1 | " | |
| | 35 | 162 | — | 0,2 | 42-е | — |
| | 36 | 161 | — | 0,2 | 60-е | |
| | 37 | 163 | — | 0,3 | 19-е | — |
| | 38 | 189 | — | 0,3 | Забита на 9-е сутки ⑬ | |
| | 39 | 126 | — | 0,5 | Выжила ⑪ | — |
| | 40 | 154 | — | 0,5 | " | |
| ⑨ Облучение и заражение | 21 | 162 | 200 | 0,05 | 14-е | + |
| | 22 | 182 | 200 | 0,05 | 12-е | + |
| | 23 | 190 | 200 | 0,1 | 20-е | ++ |
| | 24 | 159 | 200 | 0,1 | 10-е | +++ |
| | 25 | 140 | 200 | 0,2 | 9-е | ++++ |
| | 26 | 163 | 200 | 0,2 | 10-е | ++++ |
| | 27 | 137 | 200 | 0,3 | 10-е | ++++ |
| | 28 | 155 | 200 | 0,3 | 9-е | ++++ |
| | 29 | 176 | 200 | 0,5 | 10-е | ++++ |
| | 30 | 166 | 200 | 0,5 | 9-е | +++ |
| ⑩ Облучение (контроль) | 41 | 190 | 200 | — | Выжила ⑪ | — |
| | 42 | 194 | 200 | — | " | — |
| | 43 | 206 | 200 | — | " | — |
| | 44 | 180 | 200 | — | " | — |
| | 45 | 183 | 200 | — | 15-е | — |
| | 46 | 192 | 200 | — | Забита на 13-е сутки ⑬ | — |
| | 47 | 160 | 200 | — | 12-е | — |
| | 48 | 184 | 200 | — | 12-е ⑬ | — |
| | 49 | 163 | 200 | — | Забита на 10-е сутки | — |
| | 50 | 175 | 200 | — | 10-е | — |

[Legend on next page]

[Legend of Table 3 on previous page]

1. Content of experiment; 2. No. of guinea pig; 3. Weight of guinea pig, grams; 4. Dose of radiation, r; 5. Dose of infection (on day of irradiation), cc; 6. Survival time, days; 7. Jaundice (the plus signs designate the degree of expression of the jaundice); 8. Infection control; 9. Irradiation and infection; 10. Irradiation control; 11. Survived; 12. Sacrificed on the ___th day.

with a dose of 200 r only one died on the 10th day; all those infected after irradiation died on the seventh day. The intensity of jaundice and hemorrhage was greatest in the last group.

Increase in the sensitivity of irradiated white mice to pathogenic leptospiras has also been described by L. I. Grigor'yev (1957, 1958).

These are some of the facts illustrating the next general rule: in radiation sickness the sensitivity of animals to microbes -- the pathogens of infectious diseases -- is markedly increased.

2. Infection with Conditionally Pathogenic Microorganisms

In recent years experimental exogenous infections caused by conditionally pathogenic microbes have been studied by very few investigators in irradiated animals. In the majority of cases the conditionally pathogenic microorganisms are utilized for creating models with the aim of studying such problems as, for example, the role of various organs in immunity (Schechmeister and others, 1955), the effectiveness of injections of tissue homogenates (Hatch, 1954), the therapeutic value of antibiotic therapy for radiation sickness (V. F. Sosova, 1959), the influence of infection on radiation injury (T. V. Kalyayeva, 1959) and others. Less direct study has been made of the infectious process caused by conditionally pathogenic bacteria. This may be explained by two factors.

First of all, by the great practical importance of studying the interaction of irradiated organisms with pathogenic microbes, the pathogens of infectious diseases. These studies, as has already been mentioned, are being conducted extensively at the present time.

Secondly, this may be explained by the quite detailed study of this problem in recent years. A large number of works previously

published established the main rules and regulations of interaction of the irradiated organism with conditionally pathogenic bacteria.

These basic rules and regulations amount to the fact that infection of irradiated animals always brings out the pathogenicity of the conditionally pathogenic microorganisms. Under these conditions, the latter cause a pathological process which in many cases is fatal. For example, intravenous injection of doses of *B. proteus* which are normally nonlethal into mice three days after irradiation (400 r) leads to an increase in the number of bacteria in the blood and death of the animals (Hatch and others, 1952). Those infected without irradiation survive, and the number of microbes in the blood rapidly decreases after injection.

Smith and coauthors (1954) utilized colon and paracolon bacilli, in addition to *B. proteus*, for infection of mice. While there were no cases of death of animals infected without irradiation and a five percent mortality in the control group, 96 percent of the experimental animals died when infected with *B. proteus* and 67 percent, when infected with colon bacilli.

Similar results were obtained in other experiments with the colon bacillus (Schechmeister and others, 1953; V. F. Sosova, 1956), as well as in experiments with *sarcina*, the Danyesz bacillus [a variety of *Salmonella*] (Kraninger, 1933), *Pseudomonas aeruginosa* (Hamond and others, 1954), pneumococcus type III non-pathogenic for rabbits (V. F. Sosova, 1956) and others.

The increased sensitivity of irradiated animals to infection with conditionally pathogenic bacteria has been illustrated very clearly in the experiments of V. F. Sosova (1956). In these experiments irradiated rabbits were infected intradermally with colon bacillus. Instead of the benign minor inflammatory process typical of normal animals, a hemorrhagic-necrotic local process developed in the irradiated animals, increased multiplication of microbes occurred in the tissues of the skin and there was bacteremia.

In experiments with the type III pneumococcus non-pathogenic for rabbits V. F. Sosova introduced this microorganism endotracheally into irradiated and control animals. In the irradiated organism this pneumococcus behaved like a pathogenic organism -- it multiplied and penetrated into the blood, which was never observed in non-irradiated rabbits. The inability of non-pathogenic microorganisms introduced endotracheally to "break through" the pulmonary barrier has been known since the time of V. K. Vysokovich. The results of other experiments can be presented, and they all attest to the fact that

defense against infections in radiation sickness is so markedly impaired that susceptibility is increased not only to infection with pathogenic but also to conditionally pathogenic bacteria.

3. Time of Increase in Sensitivity to Infection

Experiments for finding out the time of increase in the sensitivity to infection after the effect of ionizing radiation can be divided into two large groups. One group of studies illustrates the immediate increase in sensitivity to infection after irradiation; the second group, the increase after a certain time, on the average equal to three days after ionizing radiation.

Increase in Sensitivity Demonstrable Immediately After Irradiation. A large group of infectious processes created in irradiated animals illustrates this principle. Naiman in 1944, infecting white rats with the *Trypanosoma lewisi*, revealed their increased sensitivity to this pathogenic microorganism an hour after irradiation of the animals. The irradiation was conducted with a dose of 300-500 r. Reduction of resistance to the infection was expressed in a more active multiplication of trypanosomes in the blood of irradiated rats. Taliaferro and coauthors (1945) infected chicks with malaria plasmodia on the day of irradiation. They also recorded the more active multiplication of parasites in the blood, greater mortality rate and a shorter lifespan of chicks irradiated with a dose of 500 r.

Of the works of recent years mention may be made of the experiments of A. A. Shevelev (1959) with tularemia; A. A. Smorodintsev (1957) and I. A. Kozlova (1958) with experimental influenza; Ye. L. Sklyanskaya (1957) with certain neurovirus infections (yellow fever, encephalomyocarditis), I. B. Beylina and L. S. Kreynina (1958) with tuberculosis.

It was shown in the experiments of A. A. Smorodintsev that infection of white mice, irradiated with a dose of 100-400 r, with the influenza virus leads to active reproduction of the pathogen in the lungs. After 10 days, the quantity of virus is two-three times greater than in control animals. I. B. Beylin and coauthors infected guinea pigs with the pathogen of the human type tuberculosis simultaneously with irradiation. Increased sensitivity of the animals was expressed in a rapid course of the tuberculosis: control guinea pigs died from the infectious disease after 57 days; irradiated guinea pigs, after 17.

In the experiments of A. S. Shevelev white mice were infected

subcutaneously with a vaccine strain of the tularemia pathogen. As is well known, the vaccine strains of this microbe possess residual virulence against mice. The infection was carried out 1.5-two hours after irradiation of the animals with a dose of 374 r. Increase in the sensitivity was expressed in a high (75-100 percent) mortality rate of the irradiated animals from tularemia with isolated cases of death in the control group.

Increased Sensitivity Demonstrable Several Days After Irradiation. In a number of cases, infection on the day of irradiation or even 24 hours or sometimes two days after it does not reveal increased sensitivity of the organism to the infectious agent. Thereby, to be sure, we do not mean low doses of radiation. We are speaking about doses of ionizing radiation which after a single effect lead to the development of the overt form of acute radiation sickness to one degree or another. Quite a few such examples have been described. Schechmeister and coauthors (1952), analyzing the problem of sensitivity of irradiated mice to infection as a function of the time after irradiation, infected the animals with the hemolytic streptococcus at various periods of time after x-ray irradiation with a dose of 360 r. It was determined that when the infection was carried out on the day of irradiation or 24 hours after it the mice were no more sensitive to the streptococcus than the controls. Only beginning with the third day did the sensitivity increase sharply. After irradiation with a dose of 200 r the increased sensitivity develops only on the sixth day. In the work of Kaplan and others (1952) an experiment with similar results has been described.

Clapper (1954) infected mice with the pneumococcus simultaneously with the irradiation in a dose of 350 r and three and six days after irradiation. In the case of infection simultaneously with irradiation the time of occurrence of bacteremia and the mortality rates of experimental and control animals were the same. In the case of infection after three-six days an increased sensitivity to the pneumococci was recorded.

Experiments performed by V. F. Sosova in 1956 on rabbits, dogs and guinea pigs also showed the presence of a certain period after irradiation during which there is no increase in the sensitivity of animals to infection with bacteria. Rabbits were irradiated with different doses, from 200 to 1100 r; guinea pigs, 500-700 r; dogs, 500 r. The infection was accomplished intradermally at various periods after irradiation. Staphylococci, streptococci, pneumococci and the colon bacillus were used. Doses of the pathogens were selected which brought

about the development of a local inflammatory process in non-irradiated animals characterized by the fact that it was eliminated rapidly, and as early as after 24-48 hours was completely walled off by a zone of proliferative inflammation. In the irradiated animals this process assumed a necrotic-hemorrhagic nature with the accumulation of tremendous numbers of microbes in the tissues and penetration of them into the blood. However, this was observed in the case of infection no sooner than two-three days after the irradiation. With infection two, 17 and 24 hours after irradiation (even lethal), the nature of the infectious process was no different from that of the control: the necrotic-hemorrhagic component in the inflammation was not predominant; the multiplication dynamics of the microbes in the tissues were the same as the figures obtained normally; bacteremia did not develop from this microbes.

Our own experiments, performed with the aim of finding out the time of occurrence of the period of increased sensitivity of irradiated animals to the pathogens of infectious diseases, can also be divided into two groups. In experiments with gas gangrene and staphylococcal infection the postradiation period, equal to several days, was recorded; during this period the animals react to the infectious agent like the normal animals. In experiments with tetanus and leptospirosis this period was not demonstrated.

In the first section of this chapter the performance of an experiment has been described, and a figure has been presented (see Fig 1) illustrating the mortality rates of white mice from gas gangrene at various periods after irradiation. Infection on the day of irradiation did not give rise to increased sensitivity. It was recorded only when the infection was carried out on the third day or later.

In Table 4 the results of intraperitoneal infection of white mice with staphylococcus aureus No 209 are presented, which was grown out on meat infusion agar. The animals were irradiated four days before the infection and on the day of infection, which was carried out simultaneously for all groups and with the same infectious material. Infection of the intact mice led to the death of 45 percent of the animals. The irradiated animals died in approximately the same numbers (48 percent) where the infection was performed on the day of the irradiation with 450 r (gamma-rays, 340 r per minute). If the mice were infected four days after the irradiation, they died in 96 percent of the cases.

When mice were infected with tetanus, and rabbits, mice and guinea pigs were infected with leptospirosis increased sensitivity to the

Table 4

Results of Intraperitoneal Infection of White Mice with Staphylococcus Aureus No 209

| ① Группа мышей | ② Число мышей | ③ Доза облучения, r | ④ Доза заражения в млрд. микробов | ⑤ Число погибших животных в последующие сутки после заражения | | | ⑥ Всего пало | ⑦ Гибель в трие суток после заражения, % |
|--|------------------|------------------------|--------------------------------------|--|----|---|-----------------|---|
| | | | | 1 | 2 | 3 | | |
| ⑧ Контроль заражения | 40 | — | 2,5 | 5 | 11 | 2 | 18 | 45 |
| ⑨ Зараженные в день облучения | 50 | 450 | 2,5 | 4 | 19 | 1 | 24 | 48 |
| ⑩ Зараженные через 4 дня после облучения | 50 | 450 | 2,5 | 44 | 2 | 2 | 48 | 96 |
| ⑪ Контроль облучения | 20 | 450 | — | 0 | 2 | 3 | 5 | 25 |

1. Group of mice; 2. Number of mice; 3. Dose of radiation, r; 4. Infecting dose in billions of microbes; 5. Number of animals which died on the days after infection; 6. Total which died; 7. Death over the three days after infection, %; 8. Infection control; 9. Infected on the day of irradiation; 10. Infected four days after irradiation; 11. Irradiation control.

pathogen was demonstrated when the infection was carried out immediately after irradiation (see the first section of this chapter, Fig 3, Tables 1, 2 and 3).

What is the reason for the existence of the two phenomena described? Why is the increased sensitivity to the infectious agent demonstrated immediately after irradiation in some cases but only after several days in others? For an answer to these questions it is necessary to analyze both groups of facts. The main principle which unites the data of each group is the duration of the infectious process. All experiments combined in the first group were performed with infectious diseases with a chronic course.

Actually, the course of trypanosomal infection (Naiman),

experimental malaria (Taliaferro and others), influenza (A. A. Smorodintsev, I. A. Kozlova) and neurovirus infections (Ye. I. Sklyanskaya) is measured in weeks. The tularemia process in the experiments of A. S. Shevelev encompassed no less than a seven-day period. In our experiments death from leptospirosis occurred on the seventh-15th day or later after infection; from tetanus, the animals died after six-10 days.

On the other hand, in all experiments recording the presence of a postradiation period during which no increased sensitivity to the infection was noted the experimenters dealt with acute infectious processes. In the experiments of Schechmeister and Kaplan acute streptococcal infection was reproduced. Experimental pneumococcal infection in mice (Clapper and others) caused the death of the animals in two-three days. Intradermal infection with different microorganisms, carried out by V. F. Sosova, provided for the development of a local process which was localized in two days; this determined the outcome of the interaction between macro- and microorganisms. In our experiments with staphylococcal infection and gas gangrene the outcome of this interaction was also decided in the first two-three days after infection. With intramuscular infection of white mice with sublethal doses of the gas gangrene pathogen or intraperitoneal injection of staphylococcus No 209, part of the animals dies after one-two or three days. Those living through this period survive, and in them the foci of infection become localized and do not progress. Recovery from the infectious process, if it does not cause death, occurs during this time. As we shall see from subsequent chapters, immunologic activity during the first few days after irradiation is largely maintained; the "break" in immunity occurs approximately beginning with the third day after median lethal doses of radiation. Therefore, for the acute infectious processes, where the outcome of the interaction between macro- and microorganisms is decided in the first two days, the fact is characteristic that the sensitivity of the animals to the infection is not changed on the day of irradiation. It increases and becomes striking beginning with the third day, that is, during the period of "break" in immunity. To be sure, what has been stated is justifiable only with respect to moderate doses of radiation leading to the development of acute radiation sickness. With doses above the lethal dose the sensitivity to infection increases sooner; with low doses, later, or it may remain unchanged.

In contrast to those described, chronic infectious processes include this period even when infection is accomplished simultaneously

with irradiation. In the case of infection with the pathogens of such infectious diseases, increased sensitivity to the pathogens is demonstrable immediately after irradiation.

If it is true that sensitivity to infection increases several days after irradiation and the results of experiments with some infectious diseases depend on the duration of the course of the infectious process, the two following rules and regulations should be observed.

First of all, immediately after irradiation the sensitivity to infection with the same pathogen should be different if a method of infection leading to the development of an acute process is used in one case and to a lingering process is used in another.

Secondly, the irradiation should aggravate the chronic infectious process even when the animal is irradiated after infection.

Both the first and the second regulations actually obtain.

The first has been shown in our experiments with gas gangrene. Intramuscular infection of white mice and guinea pigs with a culture of the pathogen in a mixture with calcium chloride, as we have seen above, leads to the development of an acute process which either causes the death of the animals or from which they recover during a period of two-three days. The sensitivity of irradiated and non-irradiated animals to infection during the first two days after the irradiation is the same. If another mode of infection is used, into a wound, leading to the development of a more chronic process, the increased mortality of the irradiated animals is observed even when the infection is performed during the first few hours after irradiation. This experiment has been described in the first section of this chapter (see Figs 1 and 2).

Something similar can be observed through a comparison of the work of P. N. Kiselev and Ye. V. Karpova (1956) with the work of G. N. Kryzhanovskiy and N. N. Lebedeva (1956). The former produced tetanus in mice by injecting low doses of toxin, which contributed to the development of a long-lasting process. The latter used high doses of toxin, which caused death of the animals after three or three-and-a-half days. The results of the first experiment showed increased sensitivity to infection immediately after irradiation, while the latter authors concluded that irradiation 10-12 minutes before injection of the toxin does not aggravate the course of tetanus intoxication.

The second regulation has been shown in the work of a number of investigators. Experiments with experimental malarial infection in chicks (Taliaferro, 1945) showed that irradiation of the animals with a dose of 500 r four, eight, 12 and 16 days after infection reduces

their survival time and increases the number of parasites in the blood. Irradiation of white mice three days after inhalational infection with the pertussis bacillus, which leads to a chronic infectious process, caused aggravation of it (B. N. Sofronov, 1956).

How long does the period of reduced resistance to pathogenic microbes last after irradiation?

Since the injurious effect of irradiation on immunity underlies this phenomenon the duration of this period must depend not only on the dose of radiation, species of animal and its individual sensitivity but also on the characteristics of the infectious process and immunity in it. Examples of different degrees of duration of this period depending on the infection, with the conditions being otherwise the same, are the following:

Our experiments showed that the normal resistance of irradiated (433 r of gamma-rays) white mice to intramuscular infection with the gas gangrene pathogen is recovered after 15 days (see Fig 1). The increased sensitivity of mice irradiated with a dose of x-rays which was equivalent to the above in its biological effect (350 r) to aerogenic infection with the hemolytic streptococcus is observed for 42 days (Schechmeister and others, 1952). After the same irradiation normalization of the sensitivity of the animals to *S. enteritidis* occurs after 30 days (Schechmeister and others, 1953); to leptospiras (see Table 2) it does not occur even for 10 weeks.

These examples show that the same species of animal irradiated with the same quantity of ionizing radiation, and in the last examples with the same dose rates (20-25 r per minute), shows increased sensitivity to different infections for various periods of time. However, during the period of overt clinical manifestations of radiation sickness it always occurs, dragging out for a long time in a number of cases.

Therefore, increased sensitivity of irradiated animals to the pathogens of infectious diseases and to conditionally pathogenic microbes occurs several days (on the average, three) after the effect of ionizing radiation and lasts for two-six weeks or even longer, depending on the dose of radiation, species of animal, and the nature of the infectious process. If the infectious process is characterized by a progressive or prolonged course, the increase sensitivity of the organism to the pathogen of this infection is demonstrable when infection is carried out simultaneously with irradiation. Increased sensitivity observed from infection of irradiated animals is an integral index of the injurious effect of radiation on immunity. An analysis of the radiation

effect on various immunity factors is given in the next chapter.

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Chapter II

BASIC IMMUNITY MECHANISMS

1. The Normal Microflora

Since the time of I. I. Mechnikov the normal body microflora and its significance in physiology and pathology of organisms have continued to be the subjects of study and discussion. The problem of this section does not include complete characterization of the normal skin, gastrointestinal tract microflora, etc. Particularly, we have not set before ourselves the task of throwing light on the status of the questions and discussions associated with this problem, because they have been presented in detail in the literature (see the monograph by L. G. Peretts, 1955; L. A. Zil'ber, 1958; the review by V. G. Geymberg, 1957, and others). In this chapter the goal has been set of characterizing changes in the body microflora in acute radiation sickness, because the constancy of the microflora is an index of the immunological reactivity of the organism and one of the factors in natural immunity (L. G. Peretts, 1955; L. A. Zil'ber, 1958). A change in the microflora can bring about the occurrence of a number of pathological processes.

At the present time, the leading part of the macroorganism in maintaining the constancy of microflora typical for each species is indisputable. This has been demonstrated through the example of the flora in the largest reservoir, the intestine, which has been particularly distinctly shown by P. N. Kiselev and coauthors (1940) and M. I. Nemenov and coauthors (1938). From their work it is seen that by acting on the vegetative nervous system [autonomic nervous system] the number of microbes in the intestine and the interrelationship between their various groups are changed. S. A. Payevskiy (1954) recorded changes in the intestinal microflora of rabbits with changes in the functional states of their nervous systems. T. B. Gorgiyev (1954) emphasizes the relationship between the composition of intestinal flora and the environmental conditions as well as the physiological state of the body. Naturally, in pathological processes occurring in the body there is an inevitable change in the quantitative and qualitative composition of the intestinal microflora (I. I. Mechnikov, 1901) and a deviation of it from the normal type, in turn, can have an unfavorable influence on a sick organism. This is natural, because the intestine is characterized not only by an abundance of bacteria -- pretenders to invasion

but also by the fact that a number of such toxic activity products of microorganisms is present in it as indole, phenols, hydrogen sulfide, ammonia, and others. Specifically for this reason very often effects directed at changing the microflora of the large intestine are therapeutically effective for various diseases (I. I. Mechnikov, 1902; L. G. Peretts, 1955).

Study of the body microflora in radiation injury deals mainly with the intestinal flora. Interest in this commensal bacterial reservoir is explained by the important part of the intestine and its contents in the pathogenesis of radiation sickness. Rapidly developing destructive processes in the intestinal mucosa, which lead to an increased permeability of its wall and bacterial invasion of the irradiated organism by the intestinal microflora, make studies of it particularly important. As for the other habitats of microbes in the body, their flora has not been studied in radiation sickness. The studies of O. G. Alekseyeva (1958) are the only material on this. She has shown that the number of microbes on the skin of dogs after irradiation increases considerably. As we shall see below, this is typical of the intestine also.

Studies of intestinal microflora after irradiation were begun long ago. Even in the 1930's, L. G. Peretts and R. S. Mostova (1933), A. Ya. Yugenburg, L. G. Peretts and R. S. Mostova (1937), and then P. N. Kiselev (1940), analysing the reasons for the general early x-ray reaction, found changes in the intestinal microflora in people after x-ray therapy. The changes which they recorded consisted of a reduction in the number of colon bacilli and an increase in the gram-positive bacteria. L. G. Peretts and his coauthors were inclined to consider these microfloral changes the reason for the early x-ray reaction. P. N. Kiselev objected to this convincingly, stating that the changes in the microflora were not the cause but rather the result of the early x-ray reaction. It should be noted that not all investigators found changes in the intestinal microflora in people after the use of therapeutic doses of radiation, even after irradiation of the abdominal region (Preissler, 1952).

Detailed and systematic study of the intestinal microflora in acute radiation sickness was made under experimental conditions. In 1949, Vincent described changes in the microflora of the small intestines of irradiated rats. He noted a considerable increase in the number of coliform bacteria and the number of pathogenic staphylococci. In 1952, Furth and coauthors studied the dynamics of the change in the number of coliform bacteria, staphylococci and streptococci in dogs'

stools after irradiation with x-rays in the LD₅₀ (450 r). For this purpose, cultures were made for two weeks before the irradiation and every three days after it. A considerable increase was found in the number of coliform bacteria and staphylococci, beginning with the third day after irradiation. In 1955, the work of Bell and coauthors and of R. V. Petrov appeared, followed by the work of B. G. Avetikyan and A. G. Artemova (1956), O. R. Nemirovich-Danchenko (1958) and A. D. Kazaryan (1958).

Bell and others described an increase in the coliform microorganisms with reduction in the number of lactobacilli in the large intestines of rats irradiated with a dose of 825 r. B. G. Avetikyan and A. G. Artemova reported an increase in the number of bacteria in the intestine of mice irradiated with a dose of 300 r.

In our experiments two groups of animals were used which initially had different intestinal microfloral compositions. Such a manner of performing the experiment made it possible graphically to demonstrate the fact that changes in the flora are the result of radiation injury to the organism and occur unitypically regardless of its original composition. For this purpose a study was made of certain indices characterizing the quantitative and qualitative composition of the intestinal microflora of irradiated animals on an ordinary diet and on a diet including lactose. As is well known, lactose added to the ordinary animal's diet brings about the transformation of the normal microflora in the intestine to the lactic acid flora (I. G. Shiller, 1952). This is caused by the fact that the lactose, because of its poor solubility and absorption as well as because of its peristalsis-increasing effect (I. A. Kopetskiy, 1900), reaches the large intestine in large quantity and creates conditions in it favorable for lactic acid bacteria (I. G. Shiller). Lactic acid bacteria become predominant in the intestine, displace the putrefactive microorganisms, delay intestinal putrefaction and reduce intoxication coming from the intestine (I. A. Kopetskiy, 1900). Therefore, on a lactose diet a microflora is established in the intestine which is different from the usual. The use of two groups of animals with different intestinal microflora before irradiation made it possible to determine the degree to which the microfloral changes after irradiation are dependent on the initial bacterial composition of the intestine and to determine whether they are caused mainly by the macroorganism.

The experiments were performed on white rats weighing from 130 to 170 grams. In each series animals of the same sex and weight were selected (130-150 grams or 150-170 grams). In all, 160 white

rats were used for the experiments. The experimental animals were observed methodically, weighed, and the microflora of the large intestine was investigated. Based on the fact that the large intestinal microflora is no different from the stool microflora (F. T. Grinbaum and E. I. Al'shtuller (1932)), we used the following method of investigation: 0.1 gram of stool was suspended in 10 cc of physiological saline solution; 0.1 cc of this suspension was transferred back to 10 cc of physiological saline solution; one drop (0.04 cc) of the latter suspension was streaked on plates of Endo agar and five percent blood (or serum) agar containing one percent glucose. From the first suspension smears were prepared and stained by the Gram method. The total number of bacteria was counted by means of counting the colonies on the plates of blood (or serum) agar; the colon bacilli were counted by counting the characteristic colonies on Endo agar. Qualitative characterization of the bacteria was made possible by isolating cultures from the colonies and by studying them with the ordinary method. For the purpose of studying anaerobic microflora an observation was made of one of the permanent representatives of the group of anaerobes in the intestine, *B. perfringens*. For isolation and count of it, the method of streaking rat stools on the surface of Wilson-Blair culture medium in Petri dishes with the subsequent pouring of meat infusion agar over it was used. After 12-18 hours of incubation of the Petri dishes at a temperature of 37-40° C a count was made of the intensely black colonies, typical of this microbe. On microscopy of the smears the interrelationship was determined between gram-negative and gram-positive microorganisms, for which purpose these forms were counted in four-five microscope fields.

These indices were studied before and after irradiation in animals which were on the usual diet (five grams of cooked meat, 17 grams of groats, 15 grams of bread) and in animals which were on the same diet to which three grams of lactose had been added, which provided for the transformation of the normal into the lactic acid microflora.

After three-four days of the lactose diet the quantitative and qualitative composition of the intestinal microflora of white rats changed markedly. The number of gram-negative elements in the smears decreased. Thus, while the average percentage of gram-negative microorganisms in smears in the case of the usual diet amounted to 87-76 percent, varying within limits of 95 and 50 percent, the average percentage of gram-negative microorganisms after the transformation amounted to 31-16 percent, varying from 50 percent to zero. In

The last cases, the smears were pure cultures of positively stained lactic acid bacteria of the *B. acidophilus* type. In the cultures a reduction was found in the number of colon bacilli with respect to the total number of bacteria plated out (51-36 percent in the case of the ordinary diet and 31-23 percent with the lactose diet). The number of *B. perfringens* was reduced by more than two times; there was also reduction in the number of *Proteus vulgaris* and strains of microbes possessing hemolytic, proteolytic, indole- and hydrogen-sulfide-forming properties. Therefore, it is evident that the lactose diet brought about a very marked change in the microbial contents of the large intestine. In all cases the "lactose" animals looked perfectly healthy and gained weight normally.

For the purpose of elucidating the changes in the intestinal microflora after irradiation, control rats which received the usual diet as well as rats receiving the usual diet to which lactose had been added were exposed to a whole body irradiation with x-rays. The basic experiments (110 animals) were performed with the utilization of a dose of 600 r. In addition, 20 rats were irradiated with a dose of 1000 r and 20 rats, with a dose of 500 r. The irradiation conditions were the following: 180 kv, 15 ma, filter of 0.5 mm Cu + 1 mm Al; FSD, 40 centimeters; dose rate, 37.5 r per minute. The untypicality of changes in the intestinal microflora after irradiation made it possible to present the dynamics of several indices of these changes for the purpose of characterizing them. In view of the individual variations normally and after irradiation we are presenting the average figures for a number of rats, without dwelling on each index for the separate animals.

In Table 5 the results of the experiment with determination of the number of colon bacilli are presented. The experiment was performed on 30 rats, which were irradiated with a dose of 600 r. From Table 5 it is seen that with the ordinary diet, as early as the first two days after irradiation, the number of colon bacilli decreases in the contents of the large intestine. Thus, prior to irradiation the colon bacilli plated out of the stool amounted to 45 percent, on the average, of the total number of bacteria plated out; the day after irradiation an average of only 25 percent was plated out. However, after several days this figure increased, reached the original and exceeded it. Study of the stool after the death of the animals showed a pure culture of colon bacilli in the majority of cases.

Similar dynamics were observed for the change in the number of colon bacilli after irradiation of animals which were given a lactose

Table 5

Number of Colon Bacilli in Percentages of Total Number of Bacteria Plated Out of Stool before and after Irradiation

| ① Диета | ② До облучения | ③ Время после облучения, сутки | | | | | ④ В день смерти |
|-----------------------|-------------------|--------------------------------|----|----|----|----|--|
| | | 1 | 4 | 6 | 9 | 11 | |
| ⑤ Обычная | 45 | 25 | 49 | 47 | 54 | 75 | ⑥ В большин- стве слу- чаев 100 ⑦ То же |
| ⑥ Лактозная | 27 | 26 | 33 | 37 | 45 | 55 | |

1. Diet; 2. Before irradiation; 3. Time after irradiation, days; 4. On the day of death; 5. Usual; 6. Lactose; 7. 100, in the majority of cases; 8. The same.

diet: against the background of the original low content of colon bacilli a subsequent increase in their number occurs.

In Table 6 the results of a study of the number of *B. perfringens* are presented, obtained from the examination of 20 rats irradiated with a dose of 600 r. From Table 6 it is seen that the number of *B. perfringens* in the stool after irradiation decreased sharply to the point of complete disappearance. However, on the day of death of the rats these microbes appeared in the intestine, although in a quantity smaller than that before irradiation. Even in this case there was a parallelism noted between the changes observed in rats with the ordinary microflora and in rats with microflora transformed into the lactic acid type.

On comparing the other microfloral indices a strict parallelism is also observed between the changes noted in both groups of animals. The number of *Proteus vulgaris* increases in the first few days after irradiation, and beginning with the fourth-sixth day it steadily decreases. The total number of bacteria in the first three-four days decreases, and then rapidly increases. The number of hemolytic, proteolytic, indole- and hydrogen-sulfide-forming strains among the inhabitants of the intestine increases after irradiation, attesting to an increase

in the pathogenic properties of the intestinal bacteria. All these changes are typical of radiation sickness in both groups of animals: in rats receiving an ordinary diet and rats receiving a lactose diet.

Table 6

Number of *E. Perfringens* in Percentages of Total Number of Bacteria Plated Out of Stool before and after Irradiation

| ① Диета | ② До облучения | ③ Время после облучения, сутки | | | | | | ④ В день смерти |
|-----------------|----------------|--------------------------------|-----|-----|-----|-----|---|-----------------|
| | | 1 | 2 | 3 | 4 | 6 | 7 | |
| ⑤ Обычная . . . | 17,3 | 1,7 | — | 0,8 | — | 4,2 | — | 2,7 |
| ⑥ Лактозная . . | 7,7 | — | 1,4 | — | 0,1 | — | 0 | 3,4 |

1-6. Same as for Table 5.

On the other hand, the indices which remain unchanged after irradiation of animals with the usual intestinal microflora remain unchanged for animals with microflora transformed into the lactic acid type, that is, a parallelism is observed in this respect.

For example, the percentage of gram-negative microorganisms in the smears remains constant after irradiation, in contrast to results of stool cultures previously presented. While the number of colon bacilli plated out of the stool decreases after irradiation and then increases sharply, the number of gram-negative microorganisms in the smears, including living and dead bacteria, does not change after irradiation, remaining within normal limits. What has been stated is illustrated by Table 7, where the results of study of the number of gram-negative microorganisms in the smears of 30 rats irradiated with a dose of 600 r are presented. We see that smears from the stools of rats with the ordinary microflora contain an average of 79 percent gram-negative microorganisms; after irradiation this figure varies around the same level. A similar phenomenon is observed in rats

whose microflora has been transformed into a lactic acid microflora; the number of gram-negative bacteria in the smears varies within the limits characteristic of non-irradiated "lactose" animals.

Table 7

The Number of Gram-Negative Microorganisms in Percentages of the Total Number of Bacteria in the Smears before and after Irradiation

| ① Диета | ② До облучения | ③ Времи после облучения, сутки | | | | |
|-----------------------|-------------------|--------------------------------|----|----|----|----|
| | | 1 | 4 | 6 | 9 | 11 |
| ④ Обычная | 79 | 78 | 75 | 82 | 75 | 70 |
| ⑤ Лактозная | 26 | 38 | 35 | 20 | 22 | 20 |

1-3. Same as Table 5; 4. Usual; 5. Lactose.

The characteristics of the changes in the intestinal microflora presented above were noted in rats irradiated with a dose of 600 r. However, the few experiments which were performed on animals with doses of 1000 and 500 r attested to the existence of microfloral changes of the same nature in these rats also.

How are we to explain the facts obtained? First of all, it is necessary to note once again that irradiation causes certain changes in the composition of living bacteria of the intestinal microflora in white rats. The nature of these changes remains constant even if the animals are irradiated with an artificially altered (in this case to the lactic acid type) intestinal microflora. This indicates that these are not accidental changes in the microflora but rather that the nature of the changes depends largely on the radiation injury to the body rather than on the quantitative or qualitative composition of the intestinal microflora.

Actually, if we analyze the data of Tables 5 and 7 once again, it may be seen that the number of colon bacilli which can be plated out (that is, living) changes in a unotypical manner in both groups of animals, despite the fact that the original index of this microorganism in

the intestine of "lactose" rats was considerably less (27 percent as against 45 percent). On the other hand, the microbial profile of the smears does not change after irradiation, that is, the number of gram-negative bacteria remains the same as in each group before irradiation. A discrepancy is obtained: judging from the smears, the number of colon bacilli (the main mass of gram-negative microorganisms) does not change in the stool, but the cultures show definite changes (original decrease and subsequent increase in the number of colon bacilli). This discrepancy can be explained only by the fact that in the smears, where living and dead microorganisms are counted, a large number of nonviable bacteria is evidently demonstrated in the initial period. This may be related to the secretion of bactericidal substances into the intestinal lumen because of destruction of the mucous membrane, which occurs in the first-second day after irradiation of the animals (N. N. Klemparskaya, 1955; N. A. Krayevskiy, 1957). N. N. Klemparskaya (1955) showed that the walls and content of the small intestines taken from irradiated animals during the period of destruction of the mucosa possess a considerable bacteriostatic effect with respect to the colon bacillus, staphylococcus and some other microbes, which is greater than normal. Apparently, directly after irradiation a number of microbes -- inhabitants of the intestine -- are under unfavorable conditions and die, the result of which is a reduction in the number of colon bacilli, *B. perfringens*, and a reduction in the total number of microbes.

With the development of radiation sickness the majority of vital functions of the organism is impaired, there is a reduction in its reactivity, and the most antagonistic species of bacteria, the colon bacillus, predominates in the intestine; there is an increase in the number of *B. perfringens* as well as in the total number of bacteria and of the pathogenic strains among them.

From an analysis of the Tables presented it follows that a lactose diet, without influencing the nature of the changes in the microflora after irradiation, reduces the magnitude and slows the rate of these changes. Thus, for example, the number of colon bacilli plated out of the stools of animals on an ordinary diet amounted to 49 percent on the fourth day; 75 percent, on the 11th day; on the same days after irradiation of the "lactose" animals 33 and 55 percent, respectively, were plated out (see Table 5). Similar results were obtained in the experiment with *B. perfringens* (see Table 6).

Data on the occurrence of a large number of hemolytic, indole- and hydrogen-sulfide-forming strains as well as strains possessing

proteolytic properties can indirectly attest to qualitative changes in the properties of microbes living in an organism affected by radiation. Direct proof of this is found in the studies of G. A. Shal'nova (1959), O. R. Nemirovich-Danchenko (1958), N. N. Klemparskaya (1959). The last two authors showed an increase in the number of bacteria with signs of antibiotic-resistance in the intestines of irradiated animals, despite the fact that there had been no contact between these bacteria and the antibiotics. G. A. Shal'nova isolated a large number of strains of the colon bacillus from irradiated and non-irradiated animals and determined the minimum lethal doses of both for mice. A statistically significant increase of the number of strains possessing more pronounced pathogenic properties was demonstrated in the intestines of irradiated animals.

* *
*

What has been presented shows that the changes occurring in the normal intestinal microflora in radiation sickness are characterized by qualitative and quantitative modifications of the composition of the microbial inhabitants. The quantitative changes consist of an increase in the total number of microbes. The qualitative changes may be divided into two types.

1. Change in the interrelationship between various representatives of the flora, for example, increase in the number of coliform bacteria and staphylococci with a reduction in the number of lactobacilli and some anaerobes.
2. The occurrence of a large number of bacteria possessing hemolytic, proteolytic, indole- and hydrogen-sulfide-forming properties as well as the occurrence of a greater than normal number of strains with altered properties, for example, antibiotic-resistance and strains with more pronounced pathogenicity.

Whether all this is evidence of variation in bacteria because of their living in an altered environment, selection of mutants, or is the result of colonization of the microfloral reservoirs with new representatives is a problem which has not been studied at the present time.

2. Tissue Permeability and the Segregating Function of the Reticulo-Endothelial System

The segregating function of the reticulo-endothelial system, it

would appear, should be regarded from the aspect of the effect of radiation on phagocytosis, since specifically the phagocytic function of this system is basic from the immunological viewpoint. We are analyzing this problem along with tissue permeability for the following reasons. Increased permeability of all biological barriers developing after the effect of ionizing radiation on the body leads to the penetration of various foreign substances into the blood, including bacteria. Increased permeability of the histo-hematic barriers also provides for increased passage of substances from the blood into the tissues. In addition, the adsorptive properties of biological structures are changed after irradiation. Specifically for this reason all these phenomena, of which the process of clearing the blood of foreign substances is made up, are expediently analyzed together, because accelerated or retarded clearance of the blood with respect to substances introduced into it does not mean activation or depression of the phagocytic power of reticulo-endothelial cells. This may depend on permeability changes or changes in the adsorptive properties of the tissues.

It should be noted that immunologists have studied the adsorptive properties of tissues of irradiated animals absolutely inadequately and have not taken into consideration the role of change in these properties in processes of the effect of radiation on immunity. In the monographs and reviews known to us this problem has not been discussed, and no analysis is given of data from the literature.

Increase in the tissue permeability after irradiation has been described repeatedly. At the present time, a study has been made of the intestinal permeability (P. N. Kiselev, 1954; V. L. Troitskiy and M. A. Tumanyan, 1958), of pulmonary (A. Ye. Ivanov and V. F. Sosova, 1954), hemato-ophthalmic (P. N. Kiselev, 1950; N. I. Arlashchenko, 1958) and hemato-encephalic barriers (M. M. Gromakovskaya, S. Ya. Rapoport, 1958), of the skin and subcutaneous tissue (P. N. Kiselev, 1954; V. M. Mastryukova, 1959), blood vessels (P. D. Gorizontov, 1954; Z. N. Nakhil'nitskaya, 1958). There is agreement in the literature -- irradiation leads to an increased tissue permeability. The most complete review of data on the effect of radiation on tissue permeability has recently been published by P. N. Kiselev and Z. N. Nakhil'nitskaya (1960).

P. N. Kiselev explains the mechanism of increased permeability as the direct and indirect depolymerizing effect of ionizing radiation on hyaluronic acid, which is the main component of tissue mucopolysaccharides, and the hyaluronic acid-hyaluronidase system. Experiments on the study of the inherent viscosity of hyaluronic acid after

Irradiation of it in vivo and in vitro confirm the author's viewpoint. However, this process in vivo is much more pronounced. This made it possible for P. N. Kiselev to assume that other mechanisms are included in this process which, unfortunately, have been little studied at present, although there are data according to which the mechanism of the increased permeability cannot be explained simply by a change in the hyaluronic acid-hyaluronidase system (L. G. Tutochkina, 1955; N. I. Arlashchenko, 1956). N. I. Arlashchenko comes to this conclusion on the basis of experiments on the study of the permeability of the hemato-ophthalmic barrier in irradiated rabbits. She showed that the administration of 50 viscosity units of hyaluronidase intravenously or subconjunctivally increases the permeability of the tissues to fluorescein, which may be judged by the acceleration of outflow of this dye from the anterior chamber of the eye and the skin of the lids. However, no acceleration or increase in the outflow of the dye from the blood into these tissues, that is, the reactions typical of irradiated animals, is observed. The author draws the substantiated conclusion that other factors aside from the hyaluronic acid-hyaluronidase system participate in mechanisms of increasing permeability.

Z. N. Nakhil'nitskaya (1958), studying the vascular permeability to fluorescein, performed experiments in which the condition of increased tissue permeability was created in rabbits not only by x-ray irradiation but also by sensitization of the animals through histamine injection. Such parallels are exceedingly interesting, since it is known that there is an accumulation of histamine and histamine-like substances in the blood of irradiated animals (P. D. Gorizontov, 1959) as well as a state of autosensitization after the effect of ionizing radiation (N. N. Klemparskaya, O. G. Alekseyeva, R. V. Petrov, V. F. Sosova, 1958). Z. N. Nakhil'nitskaya showed that the curve characterizing the outflow of dye from the blood of irradiated animals is specific for them. In states of increased vascular permeability brought about by sensitization or injection of histamine, the dynamics of the dye outflow are different. A very important part is apparently played by the vitamin balance, since the administration of vitamin P normalizes, to a great extent, the irradiation-increased permeability (P. N. Kiselev, P. A. Buzin, 1956). The facts presented permit us to state that the mechanism of increase in tissue permeability after the effect of ionizing radiation on the body is a very complicated one and cannot be related to any single factor.

In studying the tissue permeability for living objects -- microbes -- consideration should be given to their invasive power and the

activity of the defense mechanisms of the macroorganism. The penetration of bacteria from the intestine into the blood, to be sure, cannot be explained simply by increased permeability of the intestinal wall. The latter is markedly increased during the first few days after irradiation and then, to a certain degree, is normalized. Nevertheless, the microbial invasion continues (see Chapter III) in connection with the fact that, in addition to increase in permeability, a depression of the phagocytic antimicrobial mechanisms is also observed in the intestinal wall (A. Ya. Fridenshteyn, 1958), with the accumulation of tremendous numbers of microbes in the intestinal lumen, qualitative changes in the microflora, and others. However, in the present chapter we shall not consider problems of microbial invasion.

The time of occurrence of increased permeability has been studied and made more precise in recent years. All investigations have confirmed the rules and regulations established by P. N. Kiselev (1954), which he obtained by means of intradermal injection of trypan blue. These rules and regulations amount to the fact that increased permeability develops rapidly, during the first day after irradiation, and is maintained for two-three days and then is normalized. N. I. Arlashchenko (1958) showed this through the example of the hemato-ophthalmic barrier; Z. N. Nakhil'nitskaya (1958), through the study of vascular permeability; M. A. Tumanyan and F. M. Sosnovskaya, through the study of penetration of antigenic substances from the intestine. Aside from this, these authors established a second wave of increase in the tissue permeability, which began a week after irradiation or shortly before death of the animals.

The majority of authors studying tissue permeability after irradiation also studied the dynamics of its changes. The data of P. N. Kiselev, N. I. Arlashchenko, Z. N. Nakhil'nitskaya and others showed the development of increased permeability as early as the first few hours after irradiation. The maximum figures for increase in tissue permeability are noted during the first two days after irradiation and in the antemortem period. Similar data have been obtained by L. S. Shtern (1958), M. M. Gromakovskaya and S. Ya. Rapoport (1958), through a study of the permeability of the histohematic barriers by means of radioactive phosphorus. Schematically, the change in tissue permeability in acute radiation sickness may be represented in the form of a double-hump curve (Fig 4).

If we plot the changes in the adsorptive properties of tissues of irradiated animals and the phagocytic activity on the schema shown, it will assist us in analyzing controversial data on the segregating power

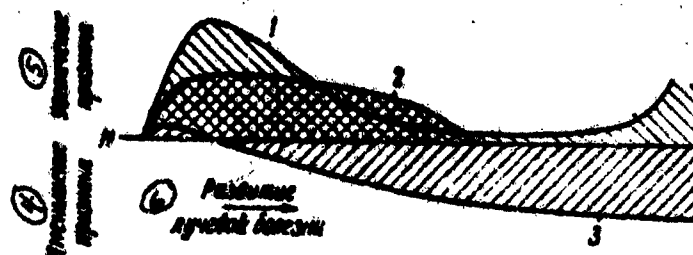


Fig 4. Schema of Changes in Tissue Permeability, Adsorptive Properties of the Tissues and Phagocytic Activity with Respect to Time in Radiation Sickness: 1. Permeability; 2. Adsorptive properties; 3. Phagocytosis; 4. Reduction of the sign; 5. Increase in the sign; 6. Development of radiation sickness.

of the reticulo-endothelial system in radiation injury.

The adsorptive properties of the tissues were studied in experiments with different models. Savitsky (1955) determined the power of leukocytes for being adsorbed on glass wool (adhesive properties). It was determined that during the first day after irradiation of dogs and guinea pigs the adhesive properties of leukocytes increase by several times and remain at an elevated level for some time. L. A. Frenkel' (1958) reported an increase in the adsorptive properties of proteins developing in the first few hours after irradiation of rats. However, as early as after three hours, a reduction in the adsorptive activity begins. M. V. Tikhomirova (1958) determined, by means of a precipitin test, the antigen (horse serum) fixation rates by different organs of normal guinea pigs and guinea pigs irradiated with a dose of 500 r. It was found that the adsorption of antigen by liver tissue increases on the first-third day after irradiation, becomes normal on the fifth-seventh day, and rises again before death (ninth day). A similar phenomenon is observed with respect to intestinal tissues; only in this case normalization occurs on the third day.

O. G. Alekseyeva (1959) studied the adsorption of staphylococci by small intestinal tissues, mesenteric lymph nodes, spleen, liver, kidneys and muscles in rabbits irradiated with a dose of 800 r.

The author notes that the greatest degree of expression of the adsorptive activity is characteristic of liver cells. In the first two days after irradiation there is an increase observed in the adsorption of microbes by these cells. The same thing applies to renal cells. In the small intestine and lymph nodes a reduction of adsorption is observed. However, since in the cells of these tissues the adsorptive activity is generally weakly expressed with respect to the staphylococcus, they are of less interest to us. After three days, normalization of the adsorptive properties of all tissues begins. M. I. Fedotova (1959) studied the adsorptive properties of tissues of the liver, small intestine and muscles of white rats irradiated with a dose of 700 r by the method of vital staining with neutral red. Unotypical variations were recorded in all tissues. In the first 24 hours there was an increase in adsorption of the dye by 120-140 percent. In the next six days the adsorptive properties of the tissues varied, but were no less than normal figures. The maximum adsorptive activity (225 percent) was recorded on the third day after irradiation. After the seventh day the adsorptive power becomes less than normal. Beginning with this day, the main mass of animals dies. The data of M. I. Fedotova are in agreement with the data of L. I. Korchak (1957) obtained through the utilization of neutral red and methylene blue.

E. Ya. Grayevskiy and M. M. Korchak (1959), studying the uptake of dyes by tissues of irradiated animals in vivo and in vitro, concluded that there was a considerable increase in the adsorptive activity in the first day after irradiation and a gradual reduction of it subsequently. In a number of tissues antemortem increase in the adsorptive properties were observed.

We, in cooperation with G. M. L'vitsyna, performed experiments on adsorption of complement by the tissues of irradiated animals (27 rats, 150 mice, 22 guinea pigs). Experiments on mice showed an increase in complement-fixation by tissues 24 hours after irradiation with x-rays in a dose of 600 r. The experimental method consisted of the determination of the minimum quantity of tissue extract possessing anticomplementarity when the working dose of guinea pig complement was added. The working dose amounted to 120 percent of the complement titer, that is, of the minimum quantity of it sufficient for assuring complete erythrocyte hemolysis under the influence of hemolytic serum.

From Table 8 it is seen that normally in the majority of cases (26 out of 46) tissue anticomplementarity is recorded in a dilution which is no higher than 1/100. Only in eight out of 46 cases is the

complement adsorbed by tissues diluted more than 1,000 times. Twenty-four hours after irradiation, the majority of experiments (19 out of 28) showed anticomplementarity of tissues diluted by more than 1/1000. On the seventh day the picture became normal. The anticomplementary activity of tissues of rats killed two days after gamma-irradiation (2,000 r) was increased. In guinea pigs an increase in tissue anticomplementarity was noted one and seven days after irradiation with x-rays in a dose of 500 r.

Table 8

Frequency of Tissue Dilutions in which They were Anticomplementary.
Radiation Dose 600 r

| ① Наименование тканей | ② Степень разведения тканевых суспензий и число положительных реакций в разных группах мышей | | | | | | | | |
|-----------------------------------|---|------------------------|--------------------|---------------------------------------|------------------------|--------------------|---------------------------------------|------------------------|--------------------|
| | ③ контроль | | | ④ через один сутки после облучения | | | ⑤ через семь суток после облучения | | |
| | ⑥ менее 100 | ⑦ от 100 до 1000 | ⑧ более 1000 | ⑥ менее 100 | ⑦ от 100 до 1000 | ⑧ более 1000 | ⑥ менее 100 | ⑦ от 100 до 1000 | ⑧ более 1000 |
| | | | | | | | | | |
| ⑨ Слизистая ки- шечника | 9 | 3 | 0 | 0 | 3 | 6 | 2 | 3 | 1 |
| ⑩ Печень | 4 | 2 | 4 | 0 | 1 | 4 | 5 | 1 | 0 |
| ⑪ Селезенка | 6 | 4 | 1 | 2 | 1 | 4 | 4 | 2 | 0 |
| ⑫ Почка | 7 | 3 | 3 | 1 | 1 | 5 | 5 | 1 | 0 |
| ⑬ Суммарное число реак- ций | 26 | 12 | 8 | 3 | 6 | 19 | 16 | 7 | 1 |

1. Name of tissue; 2. Degree of dilution of tissue suspensions and the number of positive tests in various groups of mice; 3. Control; 4. 24 hours after irradiation; 5. Seven days after irradiation; 6. Less than 100; 7. From 100 to 1,000; 8. More than 1,000; 9. Mucous membrane of intestine; 10. Liver; 11. Spleen; 12. Kidney; 13. Total number of reactions.

Summing up all the studies of adsorptive properties of tissues, it may be noted that they are increased during the first 24 hours after irradiation and then become normal at a rate different for different tissues and objects. On our diagram (see Fig 4) the changes in the adsorptive activity of tissues of irradiated animals may be represented in the form of a curve lying above the normal values throughout the greater portion of the course of radiation sickness. Data are extremely sparse on antemortem increase (M. V. Tikhomirova, 1958; E. Ya. Grayevskiy, 1959) or decrease (M. I. Fedotova, 1959) in the adsorptive activity of tissues.

It remains for us to plot the last main component determining the segregating function of the reticulo-endothelial system on the diagram -- the phagocytic activity of cells in radiation sickness. The majority of studies indicates a certain brief (first few hours after irradiation) activation of phagocytosis with a subsequent progressively deeper depression of it (see section 4 of the present chapter).

The schema presented is very relative, to be sure. It illustrates only the direction of the three characteristics of interest to us in the case of utilization of some minimum lethal dose of radiation and does not at all provide for variations undoubtedly encountered with various doses of radiation and in different animals. Nevertheless, analysis of this schema makes it possible to state that clearance of foreign admixtures from the blood stream may not be the same in different periods of radiation sickness, depending on the relative importance of one of the three main segregation factors. The total effectiveness of removal of foreign substances from the blood during the initial period of radiation injury may be not only unchanged but even increased because of an increased permeability of histo-hematic barriers and of the adsorptive power of tissues. However, with the development of radiation sickness, when there is an increase in inhibition of phagocytosis and permeability and adsorptive properties of tissues are reduced, the segregating power of the reticulo-endothelial system is decreased.

In connection with what has been stated the controversial data of different authors may be explained.

Callaway and Kerby (1951) found no changes in the power of eliminating staphylococci from the blood after they had been injected intravenously into rabbits at various periods after irradiation with a dose of 800 r. For the rabbits this dose was approximately the LD_{50/30}. Barrow and coauthors (1951), in experiments on rabbits, did not find an inhibition of the power of elimination of radiogold from the blood seven

[Days after irradiation with a dose of 800 r. Taplin and others (1952)] established the fact that removal of intravenously injected bacteria (*B. prodigiosus*) from the blood is suppressed in rabbits irradiated with a dose of 800 r but not in those irradiated with 300 r. Gordon and coauthors (1955) injected type A pneumococci intravenously into irradiated rabbits. In the first eight days the degree of clearance of the blood, if measured four hours after injection of the cocci, was the same in normal and irradiated animals. If the observation of the number of microbes in the blood was made for more than four hours, a very significant picture was found. During the first three days there were no differences between the two groups of animals. Beginning with the third day, the number of microbes in the blood of irradiated animals increases until death occurs. This is explained by the fact that there is an impairment of phagocytosis of microbes; they are engulfed but are not digested, and they begin to multiply.

V. P. Fedotov (1957) studied the barrier function of the liver in dogs with acute radiation sickness caused by administration of polonium. Colloidal radiogold was injected intravenously. The barrier function of the liver was evaluated by means of comparison of the radioactivities of portions of blood entering and leaving the liver obtained simultaneously. For this purpose cutaneous angiotomy cannulas were placed in the portal and hepatic veins by the method of I. A. Pigalev. Thereby, it was determined that the intensity of retention of colloidal gold by the liver during radiation sickness remains unchanged for a long time and decreases only in the last three days of the dogs' lives.

Gyi and Marcus (1957) studied the segregation of colloidal ThO_2 by the spleen after intravenous injection of it into mice irradiated with doses of 300-550 r. It was determined that two days after irradiation the uptake of thorotrast by the spleen is the same as normal. After seven days it is somewhat retarded in the group of mice irradiated with a dose of 400 r and is suppressed with higher doses of radiation, particularly after 550 r (by four-six times).

We observed the prolonged preservation of the segregating power of the reticulo-endothelial system in radiation sickness through the example of a quantitative study of autoinfection in irradiated white rats (R. V. Petrov, 1957). Animals weighing 180-200 grams were exposed to a whole body irradiation with x-rays under the following conditions: dose 600 r, voltage 180 kv, current 15 ma with a filter of 0.5 mm Cu and 1.0 mm Al. The FSD was 40 centimeters; the dose rate, 31.6 r per minute.

[On the day of irradiation, one, two, etc. up to 10 days after]

Irradiation, the rats were killed by means of ether. Immediately after this, a mesenteric lymph node and the spleen were extracted from the abdominal cavity and were placed separately in test tubes containing 4.5 cc of physiological saline solution. The organs were ground up with a homogenizer of our design (R. V. Petrov, 1956). After this, 0.5 cc of emulsion of each organ was streaked on the following nutrient media: meat infusion agar in Petri dishes; Wilson-Blair medium in Petri dishes, and four cc of semiliquid (0.15 percent) agar in test tubes. After drying of the surface the streaked Wilson-Blair medium was poured over with a layer of agar, which assured anaerobic growth conditions. After careful mixing, 0.5 cc of the contents of the test tube containing the culture was transferred to a second test tube containing 4.5 cc of the same medium; from the second, into a third, etc., up to the seventh. Therefore, the tissue dilutions obtained were from 10^{-2} to 10^{-8} . The semiliquid agar was used because the majority of aerobic and anaerobic bacteria are cultivated in it successfully, and in this medium the degree of seeding of the organs may be determined by the titration method.

Blood was taken from the heart, and cultures were made similar to the manner of culturing organ suspensions. Every day, five animals were sacrificed. The results were checked after 48 hours of incubation of the cultures at a temperature of 37°C ; the number of colonies was counted on a Petri dish containing meat infusion agar and the number of intensely black colonies were counted on Wilson-Blair medium. The black colonies were studied selectively from the viewpoint of biochemical and pathogenic properties, and their identity with *B. perfringens* was established. However, we did not attempt to give a qualitative characterization of the microbes isolated, and cultures from the Petri dish were only a second control method for the results of titration in semiliquid agar; the results were recorded from the last test tube in which the cultures showed growth. The titration method was always more sensitive and gave more constant results.

In Table 9 data are presented obtained from cultures of organs on solid nutrient media. Figures are presented representing the sum of colonies grown out on meat infusion agar and on Wilson-Blair medium. From the Table it is seen that the seeding of mesenteric lymph nodes occurred as early as two days after irradiation; that of the spleen, three days after irradiation, and it was possible to plate bacteria out of the blood only after four-five days. The degree of seeding was different at different times after irradiation. Thus, in the mesenteric

lymph nodes it increases from the second through the third day, and then decreases somewhat from the fourth to the seventh day, and increases sharply on the eighth-10th day.

Table 9

Number of Colonies of Microorganisms Grown Out on Solid Media in the Study of White Rats Irradiated with a Dose of 600 r

| ① Обследуемая ткань | ⑤ № крысы | ⑥ Время, прошедшее от момента облучения до забоя, сутки | | | | | | | | | | |
|--------------------------------|-----------------|---|---|-----|-----|-----|-----|-----|----|-----|-----|-----|
| | | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| ② Мезентерияль- ный узел | 1 | — | — | — | 204 | — | 1 | — | 24 | 320 | 626 | 600 |
| | 2 | — | — | 7 | 4 | 20 | 15 | 963 | 20 | 85 | — | — |
| | 3 | — | — | 102 | 12 | 179 | 39 | — | 41 | — | 73 | — |
| | 4 | — | — | 11 | 112 | 1 | 5 | 5 | 50 | 126 | 54 | — |
| | 5 | — | — | 28 | 2 | 106 | 12 | 10 | 12 | — | 101 | — |
| ③ Селезенка | 1 | — | — | — | 579 | 11 | 474 | 172 | 1 | 380 | 1 | 267 |
| | 2 | — | — | — | 3 | 33 | 18 | 14 | 40 | 267 | — | — |
| | 3 | — | — | — | 6 | 1 | — | 921 | 48 | 318 | — | — |
| | 4 | — | — | — | 60 | 1 | 22 | 15 | 17 | 592 | 513 | — |
| | 5 | — | — | — | 3 | 1 | 338 | 100 | 5 | — | 110 | — |
| ④ Кровь | 1 | — | — | — | — | — | — | — | — | 5 | 1 | — |
| | 2 | — | — | — | — | — | 260 | — | 4 | — | — | 9 |
| | 3 | — | — | — | — | — | — | 1 | — | 1 | 7 | — |
| | 4 | — | — | — | — | 1 | — | 3 | 14 | 1 | 1 | — |
| | 5 | — | — | — | — | — | 51 | — | 3 | 1 | — | — |

1. Tissue investigated; 2. Mesenteric lymph nodes; 3. Spleen;
4. Blood; 5. Number of rats; 6. Time between irradiation and
sacrifice of the animal, days.

From the third through the seventh day after irradiation the seeding of the spleen does not increase. Beginning with the eighth day, the number of bacteria isolated increases just as much as in the mesenteric lymph node. The number of microbes in the blood remains at low figures until the seventh day.

More significant results for the blood were obtained by means of the titration culture method in semiliquid agar (Fig 5). Despite the individual spread, changes in the degree of tissue seeding similar to those described above are seen distinctly.

Agreement between the results obtained by the two methods permits us to speak of their reliability. These results showed that the penetration of bacteria from the intestine into the blood begins latest, starting with the third day after irradiation at which time they are first found in the spleen. However, in the majority of cases it is impossible to find them in the blood until the seventh day. However, isolated microbes can be plated out of the blood even later, whereas hundreds of them are obtained from the spleen. Therefore, the segregating function of the reticulo-endothelial system is preserved. Only on the ninth-10th day are the mechanisms of blood clearance completely impaired -- a large number of microbes is isolated from the blood, and in a number of cases solid growth is found on the Petri dishes.

N. N. Klemparskaya (1959), studying the mechanisms of development of endogenous infection in irradiated mice, worked out an original and very demonstrative method. The author utilized the phenomenon of bacterial penetration into the blood when a massive dose of them is introduced into the gastrointestinal tract. Healthy mice and mice irradiated with a dose of 600 r were given 1,000,000,000 typhoid bacteria orally at different times after irradiation. An hour after introduction of the microbes the animals were killed. The frequency with which bacteria were demonstrated in different organs was determined (spleen, mesenteric lymph node, kidney), and a determination of the degree to which they were seeded was also made. In this way, the integral result of the segregating power of reticulo-endothelial tissues, made up of the degrees of permeability, the adsorptive and phagocytic activities of the tissues, was determined. It was established that during the first two and on the third day after irradiation the frequency and the number of microbes found in these tissues exceeded the control figures. A reduction in the degree of capture of bacteria penetrating into the blood by these tissues, compared with the normal, was recorded on the fifth-sixth day. During this period death of the irradiated mice begins. Therefore, the increase in tissue permeability developing

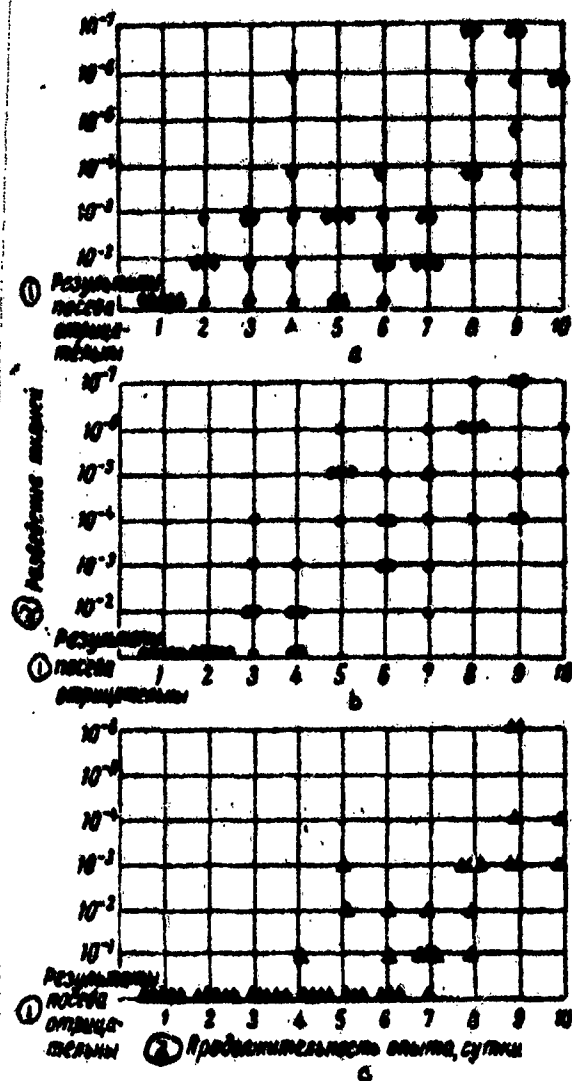


Fig 5. The Results of Titration Cultures of Rat Tissues at Various Periods after Irradiation with a Dose of 600 r: a. Mesenteric lymph node; b. Spleen; c. Blood. 1. Results of culture negative; 2. Dilution of tissues; 3. Duration of experiment, days.

Extremely quickly after the irradiation of the organism contributes to the penetration of foreign substances, including bacteria and their products into the blood. In addition, the process of passage of foreign substances from the blood into the tissues is accelerated. Increased adsorptive activity of the majority of tissues, also developing quickly after irradiation, contributes even more to the latter. The combination of these two processes in a number of cases provides for an even more effective elimination of foreign agents from the blood than normal, despite the depression of the phagocytic power of cells of the macrophage system.

There is no reason for supposing that increase in adsorption is a compensatory mechanism for the depressed phagocytosis. Apparently, the great adsorptive activity is the result of disaggregation of a number of structures and increase in the permeability of cell membranes. Incidentally, increased adsorption under conditions of radiation injury cannot be a factor in resistance, because phagocytosis of adsorbed bacteria is suppressed. However, these phenomena can lead to incorrect interpretation of the results of study of elimination of foreign substances from the blood under conditions of radiation sickness.

For a long time after irradiation this process is characterized by normal figures which are far from attesting, however, to a state of well-being in the organism but are rather the result of a series of pathological changes: the latter consist of a rapidly developing increase in tissue permeability and in the adsorptive activity and a gradually developing depression of phagocytosis. Reduction of the segregating function of the reticulo-endothelial system is found only when increase in the permeability and adsorptive activity is reduced and depression of phagocytosis is progressing.

The relationships between the significances of each of these three components in the barrier functions of separate organs determines the speed of injury to these barriers after radiation injury. For example, increase in the permeability of the histo-hematic barrier and increase in the liver tissue adsorption after irradiation cannot reduce the barrier function of the liver, because both these processes contribute to the passage of foreign substances from the blood into the tissue and can, so to speak, compensate for the depression in phagocytosis. A prolonged preservation of the barrier role of the liver actually does occur in radiation sickness (V. P. Fedotov, 1958).

Conversely, increase in the permeability of the subcutaneous connective tissue or lymph nodes contributes to the spread of microbes

for other foreign agents which have entered them, reducing their barrier role, which is observed experimentally also (P. N. Kiselev, 1954).

3. Bactericidal Properties of the Tissues

The bactericidal properties of the tissues of irradiated animals have been described repeatedly. However, every year progressively newer works are appearing on the study of bactericidal systems in radiation injury. Nevertheless, these systems have been far from completely studied at the present time even normally.

All work on the study of the bactericidal power of the tissues of irradiated animals can be divided into two groups. The first is on the study of the total bactericidal power of tissues and fluids of the body; the second, on the study of various bactericidal systems, such as complement, lysozyme, properdin and others. Study of the bactericidal activity of the intact skin and the bacteriostatic properties of extracts of internal organs of animals in radiation sickness was begun by N. N. Klemparskaya (1955). She found a rapidly occurring depression of the bactericidal power of the skin after irradiation of rabbits with a dose of 400, 800 and 1,110 r. As early as after two days, and in some parts of the body (abdominal skin) after 24 hours, a reduction of the bactericidal power is recorded. Then, the bactericidal activity varies in a wave form at levels which in the majority of cases are below normal. In subsequent years, the bactericidal power of the skin in radiation sickness was studied in different animals by a number of our and N. N. Klemparskaya's co-workers (see Chapter III). In other institutes and abroad a study has been made chiefly on the bactericidal activity of the blood, serum and some tissues.

Fishman and Schechmeister (1955) studied the bactericidal power of extracts of leukocytes obtained from rats 24 hours and three days after irradiation with a dose of 600 r. A reduction in the bactericidal factor of the extract was found after three days. Some studies were also made of this factor. Apparently, this is a protein enzyme, because it is thermolabile, not dialyzable, has an optimum pH of 7.5, and is precipitated by ammonium sulfate. N. N. Klemparskaya studied the bacteriostatic effect of extracts of tissues of the liver, spleen, lungs, intestinal mucosa and other organs of irradiated rabbits, guinea pigs and mice. The bacteriostatic influence was determined by means of adding freshly ground tissues to melted agar which had been cooled to 45° C with subsequent cultivation of bacteria on it (colon bacillus, staphylococcus). No appreciable effect of irradiation in lethal doses of

the studied properties of all the tissues was observed, with the exception of the small intestinal mucosa. Its bacteriostatic effect was increased during periods of marked breakdown and autolysis of this tissue after irradiation.

The bactericidal power of whole blood and serum was studied by Awataguchi (1957) with respect to the colon bacillus and the typhoid pathogens. A depression of this property, not associated with leukopenia, was found after irradiation of the rabbits with doses of 400-1200 r.

Marcus and Donaldson in 1953 described a reduction in the bactericidal power of the sera of rabbits irradiated with doses of 500-700 r. In 1958, they made a unique analysis of this phenomenon. It was determined that after the intravenous injection of heparin into rats or after the addition of this preparation to the blood in vitro a reduction of the bactericidal activity of the blood and serum occurs with respect to the *Bacillus subtilis*. Thereby, the effect of heparin is not associated with complement, because it has no influence on its titer. Intravenous injection of protamine eliminates the heparin effect. It might be supposed that the depression of the bactericidal power of the serum after irradiation is associated with the accumulation of heparin in the body. However, the authors showed that this is not so: the injection of protamine into irradiated rats (700 r) in different experimental arrangements did not restore the bactericidal power of the blood.

P. N. Kiselev and coauthors (1956, 1957), Fishman, Schechmeister (1955) and Kornfeld and others (1960), like the previous authors, showed that the reduction of the bactericidal strength of the serum occurs quickly, on the second-fourth day after irradiation. The maximum reduction occurs during the second week after irradiation, that is, during the period of development of autoinfectious processes (P. N. Kiselev, V. P. Sivertseva, P. A. Buzini, 1955).

Studies of the separate bactericidal systems in radiation sickness have been made on complement, lysozyme and properdin. Experiments for the determination of the quantity of complement in the blood of irradiated animals showed that the reduction of its bactericidal activity cannot be related to this factor. The data of P. N. Kiselev and coauthors (1955, 1957) and Kornfeld (1957) attest to a normal complement level in the sera of animals irradiated with lethal doses. Pillemer and others (1954) even described an increase in the complement content of the blood of rats after irradiation (500 r). Loss of the strength of the complement is observed only before death of the

animal from radiation sickness.

Reduction of lysozyme in the tissues (lungs, spleen) in the early periods of acute radiation sickness was found by Bernardini (1954). The author is inclined to explain this as the direct effect of radiation on the lysozyme. In connection with this, the observations of Caputo and Dose (1957) are very interesting; they made an in vitro study of the stability of isolated proteins and peptides to x-ray irradiation. On the basis of studies made with the aid of electrophoresis and ultracentrifugation the greatest variability of lysozyme specifically was established.

Study of the properdin system in radiation injury was begun immediately after it was discovered. The first work with irradiated animals was done by scientists who discovered this protein (Pillemer, Blum, Lepow and others, 1954; Ross and others, 1955). In experiments on rats and mice they established two basic rules and regulations: a rapid reduction in the properdin level in the blood after irradiation and the therapeutic effect of intravenous injection of purified properdin preparations. They brought about a considerable reduction in the mortality rate of animals irradiated with LD_{100/30} doses by the injection of 250 units of purified bovine properdin into each.

In recent years, a series of studies has appeared in which an investigation was made of the properdin level in the blood of irradiated animals and a study was made of the therapeutic effect of this preparation. Linder (1957) described experiments with irradiation of rats in a dose of 500 r. As early as after 24 hours a certain reduction in the properdin level in the blood was recorded (93 percent); after three days its level amounted to 52 percent; after eight days, 34 percent. Gradual recovery began on the 13th day.

Yancsura (1958) noted that 48 hours after irradiation of rats with a dose of 1500 r the quantity of properdin in the blood decreases from 20 units to 3.5 units; on the fifth-10th day, to 0.5 unit.

L. L. Chertkov, M. O. Raushenbakh, R. A. Rutberg (1957), L. L. Chertkov and R. A. Rutberg (1957), P. I. Remesov and S. D. Yakovleva (1960) determined the properdin level in acute radiation sickness in mice, rats and dogs. A reduction or complete disappearance of it from the blood was noted in cases of sickness with a fatal outcome.

The problem of the therapeutic effect of the properdin preparation in radiation sickness was studied once again by I. A. Pelishenko and coauthors (1958). The experiments were performed on white mice and rats irradiated with x-rays. Properdin was injected intraperiton-

orally or intravenously. An increase in the bactericidal power of the serum, a normalizing influence on hematopoiesis and on the blood coagulation system, as well as an increase in the survival rate of irradiated animals were noted. Afterwards, A. A. Bagdasarov, M. O. Raushenbakh, I. L. Chertkov and G. A. Chernov (1959) showed that properdin synthesis is not impaired in radiation sickness. Reduction of its quantity in the blood is apparently explained by the fact that it, possessing a great affinity for mucopolysaccharides, is bound by them. The increase in mucopolysaccharides in the blood after radiation injury is well known (L. T. Tutochkina, 1957). The effect of polysaccharides on the course of radiation sickness characterizes the prophylactic effect of this class of compounds in radiation injury, which was demonstrated by M. V. Svyatukhin and coauthors (1960). It is very possible that by binding properdin polysaccharides lead to a subsequent compensatory increase in its production.

The effect of radiation on other bactericidal blood systems has not been studied. We have not encountered any data about the change in the thermostable factor, known by the name of beta-lysine, in radiation sickness in the literature. Nor has a study been made of the factor described by Wedgewood (1958). This factor exerts a bactericidal effect on a large group of microorganisms. It provides for the activity of serum deprived of properdin, is thermolabile but is not complement, and does not require magnesium or calcium ions for its effect.

In the work of Fishman and Schechmeister (1955) an attempt was made to determine the quantity of unknown thermostable bactericidal substance in the blood of irradiated rats. They found that with a considerable reduction in the bactericidal power of the serum there is a thermostable substance the quantity of which increases. This was demonstrated through a study of serum from which the complement had been removed, that is, serum heated at 56° C. It was shown that such serum from irradiated animals is more bactericidal for *M. aureus* than de complemented control rat serum. The bactericidal power disappeared only after heating at 78° C. Therefore, an increase in beta-lysine or of some other unknown factor which is not complement or properdin was recorded. It is very possible that this is associated with the suppression of the inhibitor of some enzyme as the result of irradiation. The nature of this factor has not been deciphered.

These are the data on the influence of radiation on the bactericidal properties of tissues existing at the present time. They show in a convincing manner the essential and early reduction of the bactericidal

power of such tissues as skin and serum in radiation sickness, reduction of the quantity of lysozyme in the tissues, early reduction of properdin from the blood to the point of complete disappearance and a considerably greater stability of the complement system -- the quantity of it decreases only before the animals die. The antimicrobial activity of various tissue extracts in radiation sickness is variously expressed: the bactericidal power of extracts of leukocytes is reduced; the bacteriostatic activity of extracts of the majority of internal organs remains unchanged, while that of the small intestinal mucosa increases in connection with the autolysis occurring there.

4. Phagocytosis

The founder of the phagocytic theory, I. I. Mechnikov, distinguished two types of phagocytic cells in the bodies of mammals: microphages and macrophages. A detailed study of the macrophage system made it possible to establish its cellular composition completely (see the monograph by L. A. Zil'ber, 1958) and to distinguish a special system which, since the time of V. K. Vysokovich, has received the name "reticulo-endothelial." The test of time has not shaken I. I. Mechnikov's theory or the expediency of dividing the phagocytes into two groups.

Analyzing the effect of ionizing radiation on phagocytosis, it is expedient to consider the effect on microphages and macrophages separately. Such a subdivision is expedient, if only because a very large number of investigations has been made on the first problem, that is, the effect of radiation on the phagocytic power of polynuclear leukocytes (microphages). At the same time, study of the effect of radiation on the phagocytic power of macrophages is represented only by isolated works.

In recent years, we and a number of investigators have made many experiments devoted to studying the phagocytic activity of leukocytes in radiation sickness. These experiments permit us to express clear-cut conclusions not only about the actual fact of inhibition of phagocytosis but also concerning the time and degree of it, the dose-effect relationship and some intrinsic mechanisms of the inhibitory effect of radiation on the phagocytic activity of leukocytes. Extensive experiments made on cats, guinea pigs, rabbits and mice have been described by P. A. Busini (1957). The phagocytic power of leukocytes with respect to *Staphylococcus aureus* has been determined in experiments in vitro and in vivo in the abdominal cavity after preliminary

creation of aseptic inflammation there. The experiments were performed at different times after irradiation with doses of 500-600 r for cats and rabbits, 350 r for guinea pigs, and 200-700 r for mice. In the first day after irradiation the studies were made after two, four, six, eight, 10, 12 and 24 hours. It was determined that after a certain activation of phagocytosis during the first few hours a progressive inhibition of this leukocyte function occurs, beginning with six hours. In experiments on cats and mice the maximum inhibition (by 10 times) is recorded after two days. The maximum inhibition in irradiated guinea pigs is found six days after irradiation. The degree of inhibition of it is the same.

Study of the phagocytic activity of the blood neutrophils in irradiated rats, made by a Czech investigator (Karpfel, 1957) gave similar results. The rats were irradiated with doses of 100, 500 and 1,000 r. After irradiation with a dose of 100 r changes in the phagocytic power were not found. With larger doses the following dynamics of the changes were observed:

1. An initial increase in the phagocytic activity which coincided in time with the shift of the differential count to the right (first-second day, depending on the dose of radiation).
2. The phase of normal activity (second-third day with 1,000 r and third-fifth day with 500 r). Incidentally, at this time, as the author emphasizes, the phagocytic strength of a unit of blood is reduced as the result of reduction in the leukocyte count.
3. Phase of reduction of activity -- the result of functional inadequacy of the leukocytes.

V. V. Demidas (1957), in experiments on guinea pigs irradiated with a dose of 200 r, also showed the wave-form nature of the change in phagocytic activity of leukocytes. Inhibition developing in the first 24 hours is replaced by a temporary normalization of activity (two-four days), and then a prolonged period of inhibition again occurs.

According to the data of all authors, normalization of the phagocytic power begins in the third week if the animal does not die.

O. G. Alekseyeva, in experiments on dogs irradiated with a dose of 600 r, recorded an inhibition of the phagocytic activity of leukocytes beginning with the third day after irradiation. In the terminal period the phagocytic activity decreased practically to zero. Similar results were obtained in experiments on dogs by O. S. Sherstneva (1959).

In the study of phagocytosis at the time of its maximum inhibi

tion the relationship between the degree of inhibition and the dose of radiation is graphically illustrated: the higher the dose the greater the depression of phagocytic activity of leukocytes. In the experiments of P. A. Buzini the phagocytic index for normal mice was equal to 10.2. Two days after irradiation of the mice with a dose of 200 r it was equal to 4.4, and with a dose of 700 r, 2.5.

We performed an experiment on dogs irradiated with different doses of radiation from 3,520 to 272 r.

The phagocytic activity of blood leukocytes was determined on the third and seventh days after irradiation by the following method. Blood taken from a vein was mixed with half its quantity of three percent sodium citrate solution and with the same quantity of a suspension of *Staphylococcus aureus* No 209. The suspension contained 1,000,000,000 microbes; it was prepared by means of washing a 24-hour agar culture off with physiological saline solution. The mixture obtained was placed in an incubator for 30 minutes at 37° C. Then smears were made from it in which the percentage of phagocytosing neutrophils was determined.

From Fig 6 it is seen that the phagocytic activity of neutrophils of irradiated dogs is considerably reduced. The experiments presented were devoted only to the phenomenology of the effect of radiation on the process of phagocytosis. In them, the problem of the success of phagocytosis, in other words, the fate of the engulfed microbes, the power of the leukocytes of irradiated animals to complete the phagocytosis by digestion of the bacteria, was not touched on at all. In precisely the same way, the problem of change in the mechanisms contributing to phagocytosis -- migratory activity of leukocytes, the role of opsonins and other humoral factors -- was not touched on.

The work of L. I. Kakurin (1959) is on the power of neutrophils of irradiated animals to complete the phagocytosis by digestion of the engulfed bacteria. Four hours after intraperitoneal injection of a sterile meat infusion bouillon into rats the animals were killed, and the exudate from the abdominal cavity was collected into a test tube. The reaction of phagocytosis was performed with the leukocytes obtained with consideration of its completeness in accordance with the modified method of V. M. Berman. The principle of the method consists of determining the power of growth of the phagocytosed bacteria (colon bacillus). Contact between the phagocytes and bacteria was for 20 minutes at 37° C. In this time, normally from 61 to 76 percent of the phagocytosed microorganisms lost the power of growth, that is, they were killed (complete phagocytosis). Six-eight hours after irradiation

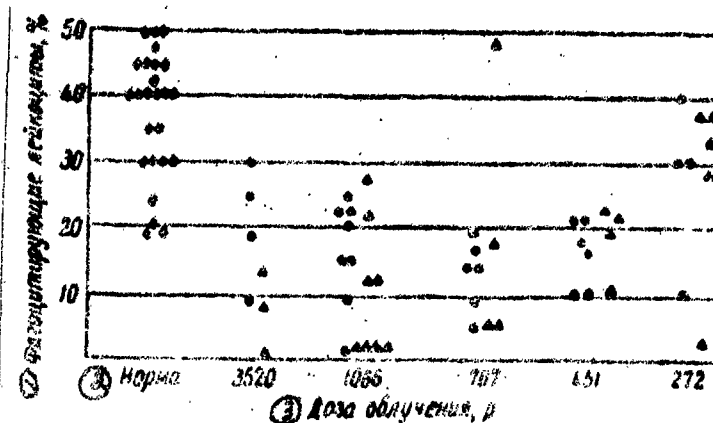


Fig 6. Phagocytic Activity of Neutrophils of the Blood of Dogs Irradiated with Different Doses: \diamond -- before irradiation; \bullet -- on the third day after irradiation; \blacktriangle -- on the seventh day after irradiation. 1. Phagocytosing leukocytes, %; 2. Normal; 3. Dose of irradiation, r.

tion of rats with a dose of 700 r, complete phagocytosis was recorded with respect to 44.7 percent, that is, it was reduced by one-and-a-half times compared with normal. These figures were obtained also in a study made five and 15 days after irradiation.

The act of phagocytosis, as is well known, depends on the presence of complement, opsonins and a number of substances which are not of immune nature in the blood, which can stimulate or depress phagocytic activity of the leukocytes or their migratory power. The latter also plays a role of quite some importance in the phagocytic process. However, at the present time information has been accumulated on each of these problems.

In the previous chapter it was shown that the quantity of complement in the blood in radiation sickness remains normal for a long time after irradiation, dropping only before the animal's death. Therefore, depression of phagocytic activity of leukocytes cannot be related to this factor. The opsonizing effect of serum after irradiation also

remains normal. In 1954, Wilkinson, testing the phagocytic activity of normal leukocytes in the presence of plasma from irradiated and non-irradiated rats, found that leukocytes from irradiated animals showed poor phagocytosis in the plasma of normal rats, while normal leukocytes possess a high degree of activity in the plasma of irradiated rats. Donaldson and coauthors (1956) concluded that the inhibition of phagocytosis does not depend on the effect of radiation on the opsonins. They used the well-known principle of increase in the opsonizing activity of serum after immunization (Taliaferro, Bloom, 1945; L. A. Zil'ber, 1958). By immunizing mice before irradiation and thereby increasing the quantity of opsonins in the blood of irradiated animals, they, nevertheless, observed the same inhibition of the phagocytic activity of the leukocytes as without immunization. In addition, they performed the reaction of phagocytosis in vitro in the presence of sera from immunized animals irradiated after immunization containing the normal quantity of antibodies. In these reactions the phagocytes were also taken from both groups of animals. The results showed an inhibition of phagocytic activity of the phagocytes from irradiated animals without regard for the humoral component and, conversely, phagocytosis by normal phagocytes was increased under the influence of immune sera of both groups of animals. Awataguchi (1957) reported the invariability of the effect of opsonins in radiation sickness of rabbits.

The data presented indicating the inessential role of disorder of humoral immunological mechanisms in the process of inhibition of phagocytosis after irradiation, do not negate the participation of other humoral mechanisms in this. Through the experiments of P. A. Buzini (1957) inhibition of the phagocytic activity of leukocytes under the influence of some substances appearing in the blood of irradiated animals was shown in a convincing manner. The nature of these substances has not been studied.

The data presented permit us to state that the main reason for depression of the phagocytic activity of leukocytes in radiation sickness does not lie in a disorder of the necessary humoral components of phagocytosis (complement, opsonins) but rather in the functional incompleteness of the phagocytes themselves -- an inhibition of the digestive power and mobility. The latter was shown through the example of the migratory activity of leukocytes (Schechmeister, Fishman, 1955). In experiments performed in vitro it was determined that the leukocytes of normal rats and rabbits possess the power of moving 0.6-0.7 millimeters in six hours. Twenty-four hours after

Irradiation the migratory power remains unchanged, but on the third, fifth, 10th and 12th days it is reduced by three-four times. The doses of irradiation for the rats were 600 r; for the rabbits, 500-800 r. The functional incompleteness of the microphages is associated with a marked reduction in their number in radiation sickness, in connection with which the integral phagocytic power of leukocytes in a certain volume of blood is reduced to an exceptional degree.

A no less essential influence is exerted by irradiation of the body on the phagocytic power of cells of the macrophagic system. Relatively little work has been published on this subject, if we do not count the studies of the total power of clearing injected foreign substances from the blood considered above. As has been shown above, this process depends on more than just the phagocytic activity of macrophages. Here, we are analyzing only those works in which tissue macrophages and their phagocytic power in radiation sickness were investigated in a morphologically precise manner. First of all, the reduction in the number of macrophages in the tissues after irradiation should be illustrated. The reduction in the number of cellular elements in the bone marrow and other hematopoietic tissues is well known (N. A. Krayevskiy, 1957). Lymph flowing out of lymph nodes contains a smaller number of lymphocytes than normal (L. A. Buldakov, 1957). The number of cellular elements in areolar connective tissue is reduced (V. V. Shikhodyrov, 1954, 1958). V. V. Shikhodyrov studied the morphologic changes occurring in areolar connective tissue in dogs irradiated with doses of 350-600 r. The animals were killed at various times, beginning with 30 minutes after irradiation. The number of macrophages in the areolar connective tissue of dogs normally amounts to approximately 20 percent of the total cell count. Two-three hours after total body irradiation the number of macrophages increases to 30-35 percent. With the development of the sickness the number of them decreases progressively, and by the third-fifth day reaches six percent. Normalization of the macrophage count is observed after three-four weeks. In the case of the animal's death the number of macrophages does not increase, but the existing cells are found at different stages of degeneration.

The functional activity of macrophages has been studied in a number of works. Donaldson and others (1956) determined the intracellular digestive power of peritoneal phagocytes. For this, normal and irradiated mice were injected intraperitoneally with a glycogen solution. After 36 hours, when the majority of cells in the peritoneal exudate was represented by macrophages, 0.5 cc of a four-percent sus-

penetration of chick erythrocytes was injected intraperitoneally. After four hours the mice were killed and the intensity of intracellular digestion was evaluated morphologically by the degree of digestion of the erythrocyte nuclei within the phagocytes. While normally digestion of 61-73 percent of the phagocytosed erythrocytes occurs in this time, after irradiation of the mice with a dose of 450 r this percentage is equal to 60 after 24 hours, 41 after six days, 39 after 18 days, and 55 percent after 22 days. A similar procedure was carried out with a different object: yeast cells were used instead of red blood cells. The normal figures amounted to 52-73 percent; two days after irradiation with a dose of 350 r, 62 percent; six days after irradiation, 49 percent; 14 days after, 47 percent; 18 days after, 54 percent. The data obtained were confirmed in experiments on rabbits (600 r).

A. Ye. Ivanov and N. N. Kurehakova (1957) studied phagocytosis in the lungs of rabbits at various periods after irradiation with a dose of 800 r. The study was made by means of the endotracheal administration of a solution of trypan blue with subsequent calculation of the phagocytic index by histological methods. Inhibition of the function of pulmonary phagocytes, which was of a phasic nature in accordance with the periods of development of acute radiation sickness, was recorded. By a similar technic a study was made of the phagocytic activity of reticulo-endothelial cells in mice with radiation sickness brought about by administration of polonium in a quantity of 0.005 microcurie per mouse (A. I. Chuchukalo, 1957). At various times after the animals were given the polonium they were injected intravenously with a one percent solution of India ink or trypan blue. The phagocytic reaction was studied on histological preparations of liver. A count was made of the number of Kupffer cells which engulfed and which did not engulf granules of the dye. Inhibition of the phagocytic activity of these cells was noted beginning with 10 days after poisoning of the animals with the emitter. The somewhat later period at which this occurs by comparison with external irradiation once is explained by the slower development of radiation injury when the animals are poisoned with the given quantities of radioactive substance.

A detailed study of phagocytic processes carried out by macrophages of the reticular stroma of the intestine in rabbits normally and in radiation sickness was made by A. Ya. Fridenshteyn (1958). It was shown that in healthy animals the microbes, penetrating from the intestinal lumen into the tissue of the lymphoid follicles, are phagocytosed by macrophages of the reticular stroma. Completion of this phagocytosis is typical for healthy animals. In the intestinal wall of

irradiated animals a reduction in the size and degeneration of the macrophages occur, and the process of phagocytosis of microbes is distinguished by its completeness. A reduction of phagocytosis and incompleteness of it are recorded from the first few days after irradiation with a dose of 800 r. Recovery occurs by the 20th day.

Therefore, in the case of an effect of ionizing radiation on animals in doses which bring about the development of acute radiation sickness a rapidly developing reduction in the number of micro- and macrophagocytes, inhibition of phagocytic activity of them and incompleteness of the phagocytic process as the result of the functional inadequacy of the phagocytosing cells, is typical of the entire phagocytic system.

5. Antibody Formation

The problem of the effect of ionizing radiation on antibody formation has been the subject of the most careful study by many scientists. Beginning with the first few years of the present century and, particularly in the past decade, a tremendous number of experiments and generalizations on this topic have been published (the reviews by I. A. Pigalev, 1955; Taliaferro, 1956; P. N. Kiselev and P. A. Buzini, 1957; the monographs by N. N. Klemparskaya and others, 1958; V. L. Troitskiy and M. A. Tumanyan, 1958). The result of these works has been the establishment of a number of major rules and regulations of the influence of radiation on antibody production.

1. Irradiation of animals with lethal or sublethal doses of ionizing radiation before immunization depresses antibody formation. This depression is most marked when the antigen is injected in the first week after irradiation, particularly on the first-second day. Recovery of normal antibody-formation in cases of survival of the animals occurs after one-two months or later.

2. The inhibitory effect of irradiation is, by and large, directly proportional to the dose of radiation.

3. Irradiation performed after immunization either has no influence on antibody production or retards it somewhat but does not prevent the accumulation of high antibody titers in the blood.

4. These data permitted Dixon (1952) and other investigators to postulate the existence of two phases of antibody formation: the initial phase is brief, associated with reception of the antigen and is radiosensitive; the subsequent phase, including the entire period of antibody production, is radioresistant. If the first phase occurs before

Irradiation, antibody formation suffers little. If the antigen is introduced after the effect of radiation, inhibition of the production of immune globulins is great.

In view of the fact that these rules and regulations are generally known and recognized, we do not have to present the literature on the basis of which they were formulated. We shall dwell in detail on a number of works of recent years, throwing light on two problems: a) whether an absolute suppression of antibody production occurs, and b) of what importance the duration of antigenic stimulation is for antibody formation in the irradiated organism. These two problems are particularly interesting to us in connection with the problem of the possibility of autoimmunization of the irradiated organism (see part 3).

A number of investigators, studying antibody production after a single immunization of irradiated animals, concluded not only that there was an inhibition of antibody production but a complete suppression of this function also. However, more careful study of this problem has shown that this is not at all so. Two main reasons for this not entirely correct conclusion may be advanced: first of all, inadequately long observation of the animals after immunization or infection; secondly, the utilization of different antigens by different authors. Inadequate duration of the observation in a number of cases was brought about by early death of animals irradiated in large doses from radiation sickness or from the infection which was studied. In such cases, the antibodies actually could not be produced, and in this sense, particularly in fatal outcomes of radiation or combined injury, the conclusion that antibodies are absent from the blood is justified. However, this does not exclude the fact that the process of antibody formation has just begun. In cases of non-fatal radiation sickness, as we shall see below, after a single immunization specifically a marked prolongation of the inductive phase occurs, that is, the time between administration of the antigen and the appearance of antibodies in the blood. The significance of the antigens utilized and the antibodies studied is important because the inequivalent suppression of the production of different antibodies is observed after irradiation. Therefore, the conclusion that there is a marked inhibition of the immunological response to some antigen or according to some antigen-antibody reaction does not mean an equally marked suppression of the production of other antibodies and cannot constitute a conclusion that the capacity of antibody formation is totally absent. Along this line the works of Makinodan and others (1957), Hummel and Battenstein (1957), Frish and Davis (1959) may be mentioned.

The first investigators immunized mice on the first, 15th and 30th days after irradiation with a dose of 475 r. As antigens sheep and rat erythrocytes were used. After immunization of control animals the agglutinin titers were the same with respect to both antigens. In the case of immunization after irradiation a marked inhibition, almost complete on the first day, of antibody formation was observed against rat erythrocytes and a considerably lesser suppression of antibody formation with respect to sheep erythrocytes.

Hummel and Battenstein immunized rabbits with human serum. The immunization was begun two hours before irradiation with a dose of 460 r. Then, at various times a study was made of the titers of cryptagglutinoids, ordinary (agglutinins) and incomplete antibodies. The irradiation did not inhibit the production of different antibodies equally by comparison with the normal. The production of cryptagglutinoids suffered most.

Frish and Davis showed that in irradiated mice the formation of hemagglutinins is depressed to a greater degree than the formation of incomplete antibodies to the same erythrocytes.

The significance of the length of the observation for solving the problem of suppression of antibody formation in the irradiated organism has been demonstrated in one of the recent works of Gengozian and Makinodan (1958). In the experiments which they described white mice irradiated with a dose of 710 r were immunized with sheep erythrocytes at different times before and after irradiation. After this, a study was made of the following: a) the duration of the inductive phase; b) the rate of appearance of antibodies; c) the highest antibody titer; d) the time the highest titer was reached, and e) the average titer for the entire period of antibody circulation in the blood, characterizing the total quantity of antibodies produced. In Table 10 data are presented on the results of postradiation immunization.

From these data it follows that with immunization at various periods after irradiation up to 45 days a considerable suppression of antibody production is observed. Judging by the highest titer, the maximum inhibition is noted when immunization is carried out 12-24 hours after irradiation; judging by the rate of appearance of the antibodies, one-five days after irradiation. However, there was no absolute suppression of antibody production in a single variant. Still, a marked increase in the inductive phase was typical of immunization in the early periods.

When immunization was performed five days after irradiation, antibodies were first recorded on the 10th day; in the case of immuniza-

Table 10

Characterization of Antibody Formation in Mice Immunized at Various Times after X-Ray Irradiation with a Dose of 710 r (Data of Gengozian and Makinodan, 1958)

| ① Время между облучением и иммунизацией | ② Критерии, характеризующие антителообразование | | | | |
|--|---|--|--|---|--|
| | ③ индуктив- ная фаза, сутки | ④ темпы появле- ния антител (lg 2 титра в сутки) | ⑤ величина наивыс- шего титра (lg 2 титра) | ⑥ время между иммунизацией и достижением максималь- ного титра, сутки | ⑦ значение среднего титра (lg 2 титра) |
| 45 мин ⑧ | 18 | 0,29 | 4,40 | 50 | 2,34 |
| 12 ч ⑨ | 13 | 0,32 | 3,80 | 50 | 2,39 |
| 1 сутки ⑩ | 11 | 0,07 | 3,60 | 40 | 2,33 |
| 5 суток | 10 | 0,08 | 5,20 | 50 | 2,90 |
| 15 » | 5 | 0,30 | 6,80 | 30 | 4,96 |
| 30 » | 3 | 0,34 | 3,70 | 50 | 5,40 |
| 45 » | 3 | 0,77 | 7,90 | 12 | 5,44 |
| Контроль ⑪ | 2 | 2,25 | 8,70 | 18 | 8,01 |

1. Time between irradiation and immunization; 2. Criteria characterizing antibody production; 3. Inductive phase, days; 4. Rate of appearance of antibodies (log 2 of the titer in days); 5. Highest titer (log 2 of the titer); 6. Time between immunization and attainment of the maximum titer, days; 7. Value of the mean titer (log 2 of the titer); 8. Minutes; 9. Hours; 10. Day(s); 11. Control.

tion 12 hours after irradiation, on the 13th day; in experiments where the antigen was administered 45 minutes after the irradiation antibodies appeared in the blood only after 18 days. However, they do appear. These data were subsequently confirmed under other irradiation and immunization conditions (Makinodan, Gengozian, 1958; Makinodan and others, 1959).

In similar experiments on rats immunized with sheep erythrocytes at various times after irradiation, Fitch and coauthors (1956) found an absolute suppression of antibody production when the antigen was administered one or six days after irradiation. The dose of irradiation

tion was equal to 500 r. However, the period of the observation was 18 days, that is, the fact that antibodies were not found may be explained by the prolongation of the inductive phase described above. This may also be the explanation for certain results of Dixon and others (Dixon, 1951) to the effect that there is a complete suppression of antibody formation when rabbits are immunized 40 hours after irradiation with a dose of 500 r.

Our own experiments (R. V. Petrov, 1957) on the study of antibody production in experimental leptospirosis in irradiated animals are in complete agreement with the data presented. The experiments were performed on 27 rabbits and 27 guinea pigs. The doses of radiation for the rabbits were 500-600 r; for guinea pigs, 200 r (RUM-3 x-ray apparatus, 180 kv, 20 ma, FSD, 60 centimeters). The "Krysa Ramenka" strain of the leptospirosis pathogen described above was used for the infection. The pathogen was grown out at 25° C on doubly distilled water to which five percent rabbit serum had been added. In the experiment cultures containing 80-100 leptospiras per microscopic field were used. The rabbits were infected intravenously with a dose of 1.5-2.0 cc of the culture. Guinea pigs were infected intraperitoneally with a dose of 0.2 cc of the culture. The infection led to the development of a latent infection without fatal outcomes in non-irradiated animals.

The results (Table 11) attest to a depression of antibody production in irradiated rabbits. This inhibition was very slight in the group of animals (Nos 1, 3, 6 and 10) infected during the first few hours after irradiation with a dose of 600 r: the onset of antibody production in the irradiated animals was delayed by one-two days by comparison with the controls. When the rabbits (Nos 39-42) were infected 24 hours after irradiation with a dose of 500 r a marked suppression of antibody production was observed: the agglutinin titer in the blood of the irradiated animals was equal to 1:40-1:1600, while the control titers were 1:400,000-1:1,600,000. The onset of antibody production was delayed by two-three days. Infection of the rabbits two days after irradiation with a dose of 600 r did not result in any appearance of antibodies in the blood.

The dynamics of antibody production and the duration of leptospiremia in rabbits of the control and experimental groups are shown in Fig 7. Therefore, a prolongation of the inductive phase and complete absence of antibodies in the case of infection two days after irradiation were found. Normally, antibodies appeared in the blood as early as on the third day after infection; in the case of infection 24 hours

Table 11

Results of Infection of Rabbits with "Krysa Ramenka" Leptospiras

| ① Опыт | ② № кролика | ③ Вес г | ④ Доза облучения р | ⑤ Срок между облучением и заражением, ч | ⑥ Длительность лептоспиремии с момента заражения, сутки | ⑦ Максимальный титр агглютинации | ⑧ Гибель животного с момента облучения, сутки |
|----------------------------|----------------|------------|-----------------------|--|--|-------------------------------------|--|
| ⑨ Контроль заражения | 2 | 2620 | — | — | 5 | 1 620 000 | Выжил |
| | 5 | 3170 | — | — | 4 | 640 000 | " |
| | 7 | 2900 | — | — | 4 | 800 000 | " |
| | 8 | 2200 | — | — | 4 | 1 600 000 | " |
| | 29 | 2800 | — | — | 4 | 800 000 | " |
| | 30 | 2900 | — | — | 3 | 400 000 | " |
| | 43 | 1610 | — | — | 3 | 800 000 | " |
| | 44 | 1530 | — | — | 3 | 1 600 000 | " |
| ⑩ Облучение и заражение | 1 | 2390 | 600 | — | 8 | 80 000 | 13 |
| | 3 | 2330 | 600 | 2-3 | 7 | 128 000 | 8 |
| | 6 | 2200 | 600 | — | 6 | 1 280 000 | Выжил |
| | 10 | 2440 | 600 | — | 6 | 1 600 000 | 13 |
| | 39 | 1860 | 500 | — | 10 | 1 600 | 13 |
| | 40 | 1530 | 500 | 24 | 10 | 320 | 13 |
| | 41 | 1620 | 500 | — | 10 | 800 | 13 |
| | 42 | 1750 | 500 | — | 9 | 40 | 10 |
| | 25 | 2810 | 600 | 48 | 7 | 0 | 9 |
| | 26 | 2580 | 600 | — | 7 | 0 | 9 |
| ⑪ Контроль облучения | 4 | 2600 | 600 | — | — | — | Выжил |
| | 9 | 2500 | 600 | — | — | — | " |
| | 11 | 2400 | 600 | — | — | — | 9 |
| | 27 | 2590 | 600 | — | — | — | Выжил |
| | 28 | 2580 | 600 | — | — | — | " |
| | 35 | 1550 | 500 | — | — | — | " |
| | 36 | 1590 | 500 | — | — | — | 28 |
| | 37 | 1610 | 500 | — | — | — | Выжил |
| | 38 | 1300 | 500 | — | — | — | " |

1. Experiment; 2. Number of rabbits; 3. Weight, grams; 4. Radiation dose, r; 5. Time between irradiation and infection, hours; 6. Duration of leptospiremia after infection, days; 7. Maximum agglutinin titer; 8. Duration of survival of animals after irradiation, days; 9. Infection control; 10. Irradiation and infection; 11. Irradiation control; 12. Survived.

after irradiation they appeared on the sixth-seventh day. From Table 11 we see that when rabbits were infected 48 hours after irradiation they died on the seventh day, that is, we could not find antibodies in the blood even if their production had begun. In experiments on guinea pigs this was confirmed. In Table 12 the results of study of guinea pigs which survived 10 and 12 days after infection 48 hours after irradiation with the absolutely lethal dose are presented. On the ninth day antibodies appeared in the blood of the animals.

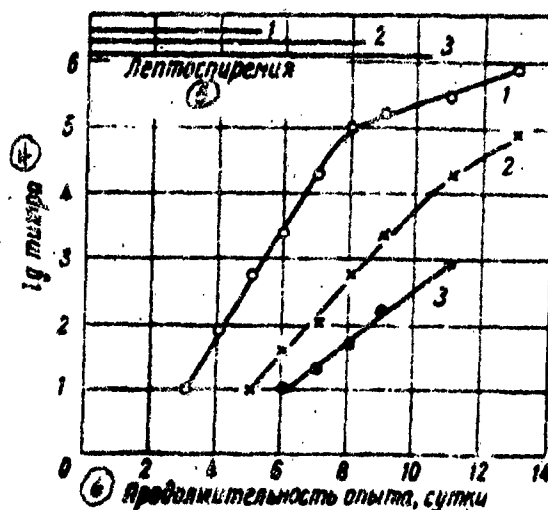


Fig 7. Antibody Titer and Duration of Leptospirosis in Rabbits Infected with the Leptospirosis Pathogen: 1. Infection control; 2. Infection two days after irradiation; 3. Infection 24 hours after irradiation; 4. Log of titer; 5. Leptospirosis; 6. Duration of experiment, days.

An analysis of the complete suppression of antibody production in mice infected with influenza virus three days after irradiation described by A. A. Smorodintsev (1957) is interesting. The absence of antibodies from the blood was recorded only in those experimental variants in which the animals died of combined injury within the first two weeks after the infection (the utilization of doses of radiation exceeding the minimum lethal dose). If the mice and rats did not die in the early periods after infection antibodies were produced, despite the

Table 12

Results of Infection of Guinea Pigs with "Krysa Ramnia" Leptospiras

| ① Опыт | ② № свинки | ③ Взр. | ④ Доза облучения мра. р | ⑤ Время между облучением и заражением, ч | ⑥ Длительность лептоспиремии, сутки | ⑦ Титры агглютинации в следующие после заражения сутки | | | | | |
|-------------------------------|---------------|-----------|-------------------------------|--|--|---|------|------|---------------|----------|-------|
| | | | | | | 4 | 7 | 9 | 11 | 14 | 17 |
| Контроль зараже- ния | 13 | 250 | | 48 | 4 | 50 | 1350 | 4000 | 4000 | 36000 | 36000 |
| | 14 | 230 | | | 4 | 50 | 1350 | 1350 | 1350 | 1350 | 36000 |
| Облучение и зара- жение | 5 | 270 | 200 | 48 | 8 | 0 | 50 | 150 | 450 | Погнб.ла | — |
| | 6 | 240 | 200 | 48 | 8 | 0 | 0 | 150 | Погнб.ла ⑩ | ⑩ | — |

1. Experiment; 2. Infection control; 3. Irradiation and infection;
4. Number of guinea pigs; 5. Weight, grams; 6. Dose of radiation,
r; 7. Time between irradiation and infection, hours; 8. Duration of
leptospirosis, days; 9. Agglutinin titers in the days following the the
infection; 10. Died.

prolongation of the inductive period, although their titers were eight-
16 times less than in non-irradiated animals.

A considerable depression but not an absolute suppression
of antibody formation after single immunizations is illustrated in the
works of P. A. Buzini (1957), V. L. Troitskiy and M. A. Tumanyan
(1958), O. P. Peterson and I. A. Koslova (1958), Taliaferro (1957)
and others.

P. A. Buzini (1957), after determining that the maximum de-
pression of antibody production is observed when antigen is adminis-
tered 48 hours after irradiation, like other investigators, performed
a series of experiments on rabbits with different antigens given at
this time. It was found that despite the marked depression of antibody
formation immune globulins are produced. Their titers, to be sure,
were much lower: for hemolysins (500 r) five times less; for agglutinins,

eight-10 times less; for precipitins (300 r), 10-20 times less. Thereby, she presents individual data for each animal, and the production of precipitins was recorded in all.

O. P. Peterson and I. A. Kozlova (1958) studied the effect of x-ray irradiation on the production of influenzal antibodies after a single vaccination of white rats. The animals were vaccinated two days after irradiation with a dose of 600 r, that is, during the period when immunization is least effective. If the authors had limited themselves to determining the antibodies in the blood only one or two weeks after the injection of antigen they would have concluded that antibody production had been completely suppressed. However, they made a longer observation and established the fact that antibodies in the blood of irradiated animals do appear but only very late, beginning with the 20th day. The antibody titers reached figures which are two-three times less than in the control rats, that is, in these experiments an inhibition and delay of the inductive phase of antibody formation occurred. Prolongation of the inductive phase of antibody formation has also been described by V. F. Sosova (1960), Ye. I. Sklyanskaya (1960), Saslaw and Cartisle (1959), O. N. Dmitriyev (1960) and others. What is this phase? In a thorough review article, Ya. Shtertsel (1959) characterizes it as follows: "The inductive phase is a real period in antibody production which differs quantitatively from the next, productive phase, primarily in its exceptional demands on metabolic conditions. During the inductive phase biochemical changes and morphologic differentiation of cells are effected." This formulation makes understandable the high degree of radiosensitivity of the inductive phase of antibody formation specifically to radiation. Actually, after irradiation in the first few hours disorders in the normal course of metabolic, particularly highly specialized processes, including protein synthesis, occur (A. M. Kuzin and N. B. Strazhevskaya, 1957; L. L. Il'ina and others, 1957; P. D. Gorizontov, 1959, and others). Impairment and suppression of all types of morphologic cell differentiation occur no less rapidly (A. P. Yegorov and V. V. Bochkarev, 1955; N. A. Krayevskiy, 1957).

Morphogenesis of certain cell structures necessary for successful antibody production is also inhibited under the influence of irradiation (Fitch and others, 1956; Wissler and others, 1958). Therefore, even in the early periods after irradiation the cells -- antibody producers -- are deprived of the biochemical and physiological capacity for carrying out processes associated with the organization of synthesis of specific globulins, that is, those processes which occur in the induc-

ductive phase. It may be supposed that prolongation of the inductive phase is explained by simple destruction of lymphoid and other cells producing antibodies. Immune globulins begin to be produced when these cells are restored and react to antigen remaining in the body. This supposition does not explain why the administration of antigen before irradiation assures antibody production after irradiation, despite cell destruction. Apparently, in addition to the reduction in the number of cells the high degree of sensitivity of the initial phase of antibody production to irradiation as a function in the accomplishment of processes of organization of antibody synthesis is of significance. This phase has been called the "radiosensitive" phase in the radiobiological literature (Dixon, 1951; V. L. Troitskiy and M. A. Tumanyan, 1958). This radiosensitivity is undoubtedly associated with the occurrence of the inductive phase.

In the case of a prolonged antigenic effect, for example, in the form of repeated or frequently repeated injections of the antigen into irradiated animals, suppression of antibody production is expressed to a lesser degree. V. L. Troitskiy and M. A. Tumanyan (1958) described experiments of triple immunization of rabbits begun two or seven days after irradiation with a dose of 600 r. The intervals between the antigen injections were equal to one week. The animals produced antibodies in titers similar to the normal.

L. G. Kovtunovich (1958) reported satisfactory antibody production by guinea pigs immunized twice three and 24 hours after irradiation with a dose of 500 r. The same author in 1960 showed the possibility of compensation for the injurious effect of radiation on antibody production by means of long-acting antigens.

Interesting experiments along this line were performed by Taliaferro and Taliaferro (1957). They irradiated rabbits daily with a dose of 125 r six times, and at various times during this prolonged sublethal irradiation they administered antigen (sheep erythrocytes) very often. The antigen was injected once and 12 times every other day. After the single administration of sheep erythrocytes in the middle of the irradiation period the inhibition of antibody production was very considerable (the rate of antibody production was 1,000 times less than normal). However, if administration of antigen every other day repeatedly was begun after three of the six irradiations, as early as the 12th day a large quantity of antibodies appeared, and with continuing injections of the antigen the antibody titers reached the normal figures. The lesser suppression of antibody production after repeated or multiple effects of the antigen can be explained by the fact

that in these cases the antigenic stimulus, covering a long period of time, comes up against cell systems which have recovered after the radiation injury and are capable of carrying out processes occurring in the inductive phase of antibody production.

Therefore, two other rules on the effect of radiation on antibody production should be added to those presented at the beginning of this section.

1. No matter how deep the inhibition of antibody production, this process is not completely suppressed. However, prolongation of the inductive phase may be so great (up to two or more weeks) that antibodies are not found in the blood as the result of the early death of animals or inadequately long observation of them. In addition, the degree of inhibition of production of various antibodies differs after immunization with various antigens.

2. Repeated or multiple injections of antigen after irradiation assure the more active antibody production by these animals compared with production of immune bodies from a single immunization.

6. Species Resistance

In view of the fact that in radiation sickness all the main resistance factors are injured the following question arises: is it not possible for irradiated animals to become sick with an infectious disease not characteristic of the given species? Tentative negative answers to this question were given long ago. Thus, Kolmer and coauthors (Kolmer, 1937) were unable to overcome the congenital resistance of rabbits, guinea pigs, rats and ferrets to the poliomyelitis virus, despite the fact that the animals were irradiated twice -- before and after infection. The dose of irradiation was equal to 100-250 r. In a report to the Thirteenth Congress of Microbiologists, O. P. Peterson and coauthors (1956) reported similar data with respect to the preservation of congenital resistance of guinea pigs to the yellow fever virus after irradiation.

In recent years, several works have been published which made a special study of the significance of the level of congenital resistance for the susceptibility of animals to infection after irradiation. First of all, mention should be made of the work of Stadler and Gowen (1957). Five genetically different strains of mice used in the experiment were characterized by different levels of congenital resistance to the pathogen of murine typhus. With respect to the degree of resistance these lines were arranged as follows: S, Z, K, Q and Ba. After

Irradiation of the mice with a dose of 320 r, the susceptibility of all lines increased; however, the sequence with respect to the degree of resistance was maintained. After irradiation with a dose of 480 r the animals of lines Ba, Q, K and Z lost their resistance completely when infected with the same doses, and the mice of line S, characterized by a high congenital resistance, maintained it in one-third of the cases. After irradiation of these mice with a dose of 640 r they lost their resistance too. Therefore, even in the case of an infection characteristic of the species the level of congenital resistance plays a great part, and in the opinion of the authors, the main part in determining the degree of increase in the sensitivity of irradiated animals to infection. A certain analogy may be drawn between this work and the work of I. A. Kozlova (1958), in which a study was made of the susceptibility of several species of irradiated animals to the influenza virus.

With respect to the degree of their natural resistance these animals are arranged in the following way: white mice are most susceptible, then come white rats, guinea pigs and rabbits. After the effect of ionising radiation in minimum lethal doses, this relationship is left intact; the greatest susceptibility was also recorded in mice; then came rats, guinea pigs and rabbits.

V. L. Troitskiy and M. A. Tumanyan (1958) infected irradiated mice with the pathogens of dysentery and typhoid. They write that despite the reduction in the natural immunity the animals did not become sick with either dysentery or typhoid. Part of the mice died but with signs of a nonspecific toxic-septic disease. It is well known that rabbits show the greatest sensitivity to dysentery pathogens of all the laboratory animals (with the exception of monkeys). After parenteral infection of them it is easy to show the enterotropism of the pathogen, hemorrhages and inflammatory changes in the large intestinal mucosa. After oral infection of bunnies with a large dose of the dysentery pathogen it is possible to produce a certain similarity in the dysenteric process to prolonged carriage of the bacilli. Therefore, the species resistance of rabbits to the dysentery pathogen is relative, and in the case of artificial infection of them after irradiation with a large dose of microbes (50×10^9) a specific dysenteric process actually develops. Dysentery never occurs spontaneously.

A similar relativity of species resistance exists in mice with respect to the pathogens of leptospirosis gryppotyphosa. A latent infectious process can be produced after artificial infection. However, attempts to produce a spontaneous epidemic by means of covering a healthy with an infected mouse end in failure. No spontaneous infection

occurs in irradiated mice either, although after artificial infection a striking infectious disease develops.

In Table 13 the arrangement and results of the experiment are shown. Two mice infected with the pathogen of leptospirosis gryppotyphosa (the "Krysa Ramenka" strain) were placed in each jar containing irradiated and non-irradiated animals. Thereby, mice were used which had been infected after irradiation with a dose of 350 r, because they excreted leptospiras for a particularly long time (see Chapter IV). After one, two and three weeks batches of the animals were killed. Their blood was studied for antibodies, and homogenates of the kidneys were cultured for isolation of leptospiras. No cases of spontaneous leptospirosis were found among the irradiated or control animals. Within the plan of the experiments presented above this experiment can also be considered evidence of the preservation of congenital resistance during radiation sickness.

Table 13

Results of Experiment in which Healthy Mice were Covered with Leptospira-Infected Mice in Jars Containing Non-Infected Irradiated and Non-Irradiated Mice

| ① № банки | Число мышей в банке ② | | ⑤ Доза радиации, р | Антитела в крови,* наблюдаемые в различное время, сутки ⑥ | | | Лептоспиры в почках*, наблюдаемые в различное время, сутки ⑦ | | |
|-----------------|--------------------------|----------------------------|-----------------------------|--|-----|-----|---|-----|-----|
| | ③ инфици- рованные | ④ неинфици- рованные | | 7 | 14 | 21 | 7 | 14 | 21 |
| | | | | | | | | | |
| 1 | 2 | 10 | 0 | +/- | | | +/- | | |
| 2 | 2 | 10 | 0 | | +/- | | | +/- | |
| 3 | 2 | 10 | 0 | | | +/- | | | +/- |
| 4 | 2 | 10 | 350 | +/- | | | +/- | | |
| 5 | 2 | 10 | 350 | | +/- | | | +/- | |
| 6 | 2 | 10 | 350 | | | +/- | | | +/- |

* В числителе (+) — результаты обследования двух инфицированных мышей; в знаменателе (-) — результаты обследования 10 неинфицированных, к которым подсажены инфицированные.

1. No of jar; 2. No of mice in the jar; 3. Infected; 4. Non-infected; 5. Dose of radiation, r; 6. Antibodies in the blood* observed at different times, days; 7. Leptospiras in the kidneys*, observed at different times, days. (*In the numerator (+) the results of study of two infected [continued next page]

[continuation of Table 13 from previous page]

mice; in the denominator (-), the results of study of 10 non-infected mice covered by the infected mice. [The word "cover" is of sexual significance here]].

We attempted to produce experimental anthrax infection in animals resistant to it, white rats. For the infection a culture of the anthrax II vaccine was used. The number of microbes used amounted to 1,000 absolutely lethal doses for mice. The infection, given subcutaneously, remained without results in all rats (10) used in the experiment. They had been irradiated three days before this with gamma-rays in a dose of 600 r. O. G. Alekseyeva did not succeed in producing diphtheria infection in irradiated rats or mice (see N. N. Klemparskaya and others, 1958).

A. S. Shevelev (1958, 1959) infected irradiated mice and guinea pigs with a vaccine strain of the tularemia pathogen. For mice it is virulent, and irradiation caused an aggravation of the infectious disease. For guinea pigs, the vaccine strain is avirulent, and a lethal infection did not develop in them even when they were infected after irradiation. Therefore, we can confidently speak of the high degree of stability of congenital resistance to the effect of ionizing radiation and the great significance of the natural resistance level in the process of increase in the animals' sensitivity to infection after irradiation. In all probability, disease not characteristic of a given species cannot occur even after irradiation. Irradiation is incapable of interfering radically with interrelationships between species of animals, on the one hand, and microorganisms, on the other, which have been built up during the course of evolution. The biochemical basis of this interrelationship needs to be studied (R. Dubosc, 1957). Irradiation does not interfere with its biochemical specificity.

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SECOND PART

INFECTIOUS PROCESSES IN THE IRRADIATED ORGANISM

Chapter III

Endogenous Infection

In the literature considerable material has been accumulated characterizing postradiation endogenous infection or "autoinfection," as it is called. Study of it was conducted along four main lines: 1) determination of the sources of endogenous infection; 2) determination of the time of bacterial invasion; 3) study of the role of endogenous infection in the pathogenesis of radiation sickness; 4) the control of endogenous infection in radiation sickness.

In analyzing the pathogenesis and significance of postradiation infection, Bond, Silverman and Cronkite (1954) wrote about the times of occurrence and roles of endogenous infection, different in principle, in the so-called intestinal postradiation syndrome ("acute intestinal radiation death") and "typical radiation death" (Osborne, 1952). The former develops after high doses of radiation, exceeding the LD₁₀₀ and is even observed after irradiation of the intestine alone. The average length of life in these cases is equal to approximately four days. Early penetration of bacteria into the blood can occur, but the significance of this phenomenon is minimal in the pathogenesis of the syndrome. The syndrome of the typical acute radiation sickness develops after doses equal to LD₁₀₀ or less. For realization of it irradiation of the entire body is necessary. The average length of life is equal to approximately 11 days. Infectious complications arise of necessity and play an important part. Radiation injury with doses above the lethal dose of radiation is of less interest to us; therefore, in the subsequent presentation, particularly in the description of the times and periods of autoinfection, the greatest attention will be given to the bacteriology of radiation injury of the second type.

1. Sources of Endogenous Infection

The problem of sources of endogenous infection has been solved by means of identification of microbes isolated from the blood in organs of irradiated animals as well as by means of direct experi-

ments, in which the routes of the bacteria from suspected sources were followed.

Study of the microorganisms isolated showed that these are normal inhabitants of the intestine or respiratory tract (Chrom, 1935; Miller and others, 1950; Bennet and others, 1951; Vogel and others, 1954; P. N. Kiselev and others, 1955; Silverman and others, 1957).

Miller and others (1950), sacrificing mice at various periods after irradiation with doses of 450-600 r of x-rays, found that the maximum number of positive cultures is obtained in the second week. They present figures characterizing the relative frequency with which various bacteria are plated out: *B. paracoli*, 42 percent; *B. coli*, 22 percent; *B. proteus*, 13 percent; *Pseudomonas*, nine percent; *alpha-streptococcus*, six percent; gram-negative unidentified bacteria, three percent; *B. alcaligenes*, two percent; anaerobes, 0.3 percent. Similar results have been obtained from blood cultures taken from dogs irradiated with doses of 350-400 r of x-rays (Bennet and others, 1951). As in the case of experiments on mice, the normal inhabitants of the intestine and respiratory tract were isolated from the blood. P. N. Kiselev and others (1955), on the basis of a study of 1100 irradiated mice and 100 guinea pigs, present the following data on the frequency with which bacteria are plated out: colon and paracolon bacilli, 55 percent; enterococci, 27 percent; staphylococci and streptococci, 11 percent; anaerobes, three percent; *B. pyocyaneus*, two percent; and *B. proteus*, one percent.

Of indubitable significance in determination of the qualitative composition of microbes which penetrate into the internal milieu of the irradiated organism is its species classification. Thus, for example, V. L. Troitskiy and M. A. Tumanyan (1955) were unable to isolate *B. perfringens* from the blood, bone marrow or spleen of rabbits irradiated with a dose of 800-1400 r. At the same time, this microbe was regularly isolated from the tissues of the internal milieu of irradiated dogs (Bennet and others, 1951) and rats (R. V. Petrov, 1957). Hammond and Miller (1955) note that in rabbits, less often than in other animals, for example, mice, bacteremia develops. In studying the blood of irradiated monkeys the relatively small number of microbial species which can be plated out attracts attention. Some investigators (Paterson, 1954, Haigh, Paterson, 1956) succeeded in isolating only *Staphylococcus aureus*, pneumococci and *B. pyocyaneus*, despite the fact that they studied 44 macaques irradiated with doses of 400-650 r. Others (V. L. Troitskiy, M. A. Tumanyan, 1958) plated out colon and paracolon bacilli, streptococci,

staphylococci and *B. proteus*. Wensinck (1958) studied postradiation bacteremia in mice of two different lines. It was determined that in the CBA line alpha-hemolytic streptococci of group G are regularly found, whereas in the C57BL line streptococci of this group are never isolated, and gram-negative bacteria are predominant.

Experiments directly indicating the fact that after the effect of ionizing radiation on the body autoinfection of it occurs with microorganisms which are inhabitants of the intestine and respiratory tract have been performed by P. N. Kiselev (1940), Bradner and others (1955), A. Ye. Ivanov and V. F. Sosova (1954). In the experiments of P. N. Kiselev irradiated rabbits were fed 24-hour cultures of *B. prodigiosus*.

Fifteen, 40 and 80 minutes after this, blood cultures were made. From seven irradiated rabbits it was possible to isolate the microbe administered orally. It should be emphasized that the blood cultures were made after preliminary block of the reticulo-endothelial system with India ink, which was injected intravenously. Without this, the blood cultures were negative. In control (non-irradiated) rabbits it was impossible to isolate microbes from the blood. These experiments indicate not only the fact that in the irradiated animals the intestinal barrier becomes permeable for microbes and the latter penetrate from the intestine into the blood but also the fact that increased permeability alone is inadequate for the occurrence of bacteremia. N. N. Klemparskaya (1959) studied the mechanisms of development of endogenous infection by means of the oral administration of massive doses of bacteria to animals. The high degree of penetrability of the bacteria administered from the intestine into the internal milieu and the greater than normal seeding of the organs and tissues with these bacteria were shown. Bradner and others (1955) isolated several species of bacteria from the blood and tissues of mice irradiated with a dose of 600 r, and then compared the antigenic properties of them with similar species of bacteria isolated from the intestine. They were identical.

In Chapter I the experiments of A. Ye. Ivanov and V. F. Sosova (1954) were presented, in which the penetration of nonpathogenic microbes introduced endotracheally through the lung barrier of irradiated animals was shown. Therefore, the normal reservoirs of bacteria (intestine, respiratory tract and apparently other places in which they live) are sources of autoinfection of the irradiated organism. It must be supposed that pathological foci of infection can also be sources of bacteremia and sepsis after irradiation.

As experimental proof of this supposition the works of Brooks and co-workers (1952), V. F. Sosova (1955), G. A. Chekatilo (1955) and V. N. Sivertseva (1955) can be mentioned. Brooks constantly observed the development of beta-streptococcal sepsis in dogs irradiated with a dose of 100 r several days after the beta-streptococcus had appeared on a burn surface. The burn was produced by application of a plate heated to 60° C simultaneously with irradiation and occupied 20 percent of the body surface. Streptococci isolated from the wound and from the blood were serologically identical. The data presented show that the source of bacteremia in radiation sickness may be wound surfaces naturally complicated by infection. The experiments of V. F. Sosova (1955) attest to the same thing for artificially created foci of infection. Thus, intradermal infection of irradiated rabbits with the colon bacillus, pneumococcus type III or *B. pyocyaneus* led to the development of bacteremia after 24 hours, which was observed until the death of the animal. If we keep in mind the fact that the number of bacteria in the skin focus does not decrease and the adsorptive power of the reticulo-endothelial cells is maintained for the first three-four days after irradiation (see Chapter II) it becomes evident that the constancy and duration of bacteremia were provided for by the continuous entrance of bacteria from the skin focus.

In experiments of V. N. Sivertseva (1955) and G. A. Chekatilo (1955) the penetration of staphylococci into the blood from inflammatory foci created in irradiated rabbits and guinea pigs was recorded.

What has been stated permits us to assert that the sources of endogenous infection in the irradiated organism are the natural bacterial reservoirs (intestine, respiratory tract, and others) as well as pathological foci of infection in the skin and other organs.

2. Times of Occurrence of Bacterial Invasion

The times of occurrence of bacterial invasion have been studied by a number of scientists (Warren, Whipple, 1923; Miller and others, 1951; Vogel and others, 1954; V. L. Troitskiy and M. A. Tumanyan, 1955; R. V. Petrov, 1956, and others). For the purpose of solving this problem all authors used methods of blood cultures or cultures of tissues of organs on nutrient media at various periods after irradiation of the animals.

In studying the blood, Warren and Whipple (1923) recorded bacteremia on the fourth day after irradiation of dogs with a lethal dose of x-rays. Vogel and others (1954) determined that the time was the same

in white mice irradiated with 170-250 reb of fast neutrons. Miller and others (1951) found bacteremia in mice, beginning with the second-third day after irradiation of them with 450-600 r of x-rays. In the experiments of V. L. Troitskiy and M. A. Tumanyan (1955) bacteria were recorded in the blood and organs of rabbits beginning with the second-fourth day after x-ray irradiation of the animals in a dose of 1100-1400 r.

Exceedingly important and important for understanding the pathogenesis of autoinfection in radiation sickness are experiments for the determination of the degree of seeding of various tissues. Such studies were made on white rats irradiated with a dose of 600 r of x-rays (R. V. Petrov, 1956). Every day, five animals were sacrificed. Blood cultures, emulsions of the spleen and mesenteric lymph node were made by the method of titration in semiliquid agar as well as on plates of solid nutrient media. This made it possible to record not only the fact of bacterial seeding but also the number of microbes in the tissues. It was found that in the mesenteric lymph node bacteria appear beginning with the second day; in the spleen, with the third; in the blood, with the fourth day. Thereby, as early as the first few days of appearance of microbes in the mesenteric lymph node and spleen the number of them in the organs is measured in tens or hundreds, and in various cases, even in thousands of individuals. Only in the blood are isolated bacteria recorded for a long time. Beginning with the eighth-ninth day the number of microbes in the tissues increases sharply, reaching tens and hundreds of thousands of individuals in the organs and, in various cases, also in the blood. The factual data of this experiment were presented entirely in Chapter II (section 2). Here, Fig 8 is presented which summarizes these data. The sequence with which the tissues are seeded, which has been described, that is, mesenteric lymph nodes, and then spleen and liver and, finally, blood, has been confirmed by the studies of Boone and others (1956). The results of the experiments make it possible to distinguish the following periods in the development of autoinfection in radiation sickness.

I. The period of sterility lasts one day and is characterized by the absence of microbes in all tissues.

II. The period of seeding of the regional lymph nodes. This period includes the time from the second through the third day after irradiation. It is characterized by the presence of bacteria in lymph nodes only.

III. The bacteremic period. It is characterized by the appearance

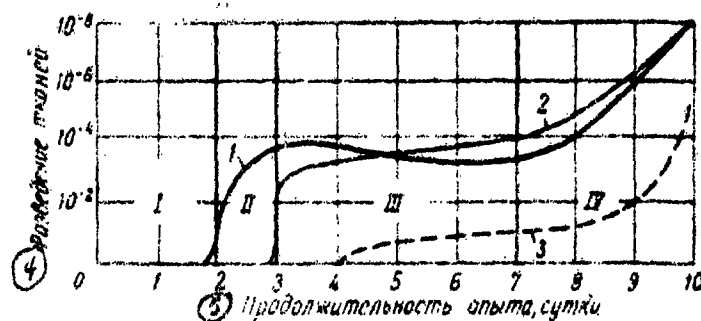


Fig 8. Dynamics of Change in the Number of Microbes in the Tissues at Various Periods after Irradiation of Rats with a Dose of 600 r: 1. Mesenteric node; 2. Spleen; 3. Blood; 4. Dilution of tissues; 5. Duration of experiment, days.

ance of a large number of microbes in the spleen. In the blood there are few bacteria, or they are not isolated at all. This period lasts from the third through the seventh day and may be called the period of relative compensation of the defense mechanisms, because the capacity of clearing the blood of bacteria has not been entirely lost, although undoubtedly a reduction of the engulfing power of the reticulo-endothelial system occurs, particularly with respect to living microorganisms (see Chapter II, section 2).

IV. The period of decompensation of the defense mechanisms. This period includes the last days (eight-10) of the animal's life and is characterized by a marked increase in the number of microbes in the organs and in the blood. Such an increase cannot be explained by an increase in the entrance of bacteria from the intestine, because the tissue permeability does not increase by comparison with that in the first few days of the disease (P. N. Kiselev, 1954). It can be explained only by the active multiplication of microbes in the tissues of the irradiated animal. The fourth period can be called, with a certain reservation, the "septic" period.

The nature of this reservation lies in the fact that the septic state occurring in the antemortem period of radiation sickness does not fit within the framework of the form of interaction of macro- and —

microorganisms which has been given the name of "sepsis." Aside from the presence of pyemia, sepsis is characterized by certain features of local and general reaction of the body, which in total define this entity as sepsis. In the irradiated organism, by virtue of its areactivity and absence of a definite local focus of infection, there are no specific reaction features typical of sepsis in the full sense of the word (septic spleen, blood reaction, and others). Specifically for this reason, in calling the fourth period "septic," we are emphasizing the relativity of this name, although in its significance for the organism the uninterrupted multiplication of microorganisms in the blood and tissues can be compared only with sepsis. A more detailed morphologic and pathologic analysis of the septic state in acute radiation sickness and sepsis has been presented in the monograph by N. A. Krayevskiy (1957).

It must be supposed that with different doses of radiation and in different animals these periods do not occur at the same time or do not occur at all. With low nonlethal doses the period of decompensation of defense mechanisms does not develop; the bacteremic period occurs later, but still occurs. For example, after irradiation of rabbits with x-rays in the region of 1100-1400 r (V. L. Troitskiy and M. A. Tumanyan, 1955) bacteremia is observed, beginning with the second-fourth day, and after 100-200 r, beginning with the fifth-eleventh day. Since the highest number of bacteria is isolated during the period of decompensation of defense mechanisms, that is, in the antemortem period, naturally the maximum number of positive cultures isolated from irradiated animals occurs on days of maximum mortality rate (Miller and others, 1950; Gonschery and others, 1953; Vogel and others, 1954; P. N. Kiselev and others, 1955). Vogel and coauthors (1956) present the following times of bacteremia in mice irradiated with x-rays in different doses: with 640 r, the 12th-14th day; with 780 r, sixth-ninth day; with 950 r, third-fifth day; with 3,000-10,000 r bacteremia is recorded after one-three days. These figures are in complete agreement with the times of maximum mortality.

If the animal does not die the bacteremic state occurs. However, the finding of microbes in the blood and tissues is observed for a long time. Bennet and others (1951), for example, in experiments on dogs irradiated with a dose of 350-450 r of x-rays, recorded bacteremia up to 28 days. V. L. Troitskiy and M. A. Tumanyan (1955) found seeding of the tissues for 23 days after irradiation of rabbits.

Therefore, with lethal or sublethal doses of ionizing radiation the onset of bacterial invasion of irradiated organisms is observed on

[the second-fourth day after the effect of radiation. Microbes are found in the blood and organs until the animal dies; in the event of recovery, for three-four weeks. The highest degree of seeding of tissues (tens and hundreds of thousands of individuals per gram) coincides with the times of maximum mortality. In the development of endogenous infection in radiation sickness four periods can be distinguished: the period of sterility, the period of seeding of regional lymph nodes, the bacteremic period or the period of relative compensation of defense mechanisms, and the septic period or period of decompensation of defense mechanisms.]

3. The Role of Endogenous Infection in the Pathogenesis of Radiation Sickness

In one of his works Bond (1957) systematizes the factual material proving the important role of infection in the pathogenesis of radiation sickness. He classifies this material in five authenticating groups.

1. Clinical observations of acute radiation sickness in people in Japan and in the United States have always revealed the existence of infectious complications in the form of anginas, ulcerative stomatitis, pneumonia, fever and others. He obtained the same data through observation of irradiated large animals.

2. The correlation in the maximum mortality rate time with the time of finding of bacteremia.

3. Data on the exceptionally increased sensitivity of irradiated animals to pathogenic and conditionally pathogenic microorganisms obtained by means of experimental infection.

4. Study of radiation sickness in animals grown under sterile conditions without microflora in the body. The experiments show that the doses of radiation necessary to kill these animals must be somewhat greater than for normal animals. With lethal doses of irradiation the length of life of sterile infection-free animals is greater than in non-sterile animals, which die with signs of autoinfection. Similar material was reported in 1955 by Talmadge.

5. Data on the effectiveness of antiinfectious therapy, particularly antibiotic therapy in radiation sickness, as well as data on the effectiveness of measures restoring the antiinfectious immunological reactivity of the irradiated organism, for example, bone marrow transplantation.

[The obviousness of the regular development and important]

role of endogenous infection in the pathogenesis of radiation sickness raises the question of evaluating this role. The question is as follows: should autoinfection be regarded as "a complication of the main disease or as a phase in its development" (N. A. Krayevskiy, 1957). In other words, is endogenous infection a necessary link in the pathogenetic chain of radiation sickness or is it only a frequent or perhaps constant complication of the main process?

For proper understanding of the role of endogenous infection in the pathogenesis of radiation sickness it is necessary to keep in mind that radiation injury can occur without autoinfection. Above, mention was made of the existence of two varieties of radiation death. The first -- "typical radiation death" -- is observed when animals are irradiated with doses of 600-1200 r, that is, with the median lethal doses of radiation (mortality rate of 50-70 percent in 11 days). Bacteremia is typical of it. The second -- "acute intestinal radiation death" -- is observed with doses above 1200 r. The mortality rate with these doses is 100 percent; the length of life for dogs is three, five days, and for rats, four or five days. Thereby, bacteremia may not develop. For example, when mice are irradiated with x-rays in a dose of 1400 r bacteremia is recorded in only 37 percent of the cases (Gonshery and others, 1953). In the case of neutron irradiation in doses leading to death in four-five days, bacteremia reaches 78 percent, but the role of this autoinfection in the pathogenesis is negligible, because antibiotic therapy, which markedly reduces autoinfection, does not change the length of life of irradiated animals (Silverman and others, 1957, 1958). On the basis of the fact that in the former case antibiotics exert a favorable effect and in the latter they do not, many authors draw the absolutely sound conclusion that the infectious factor plays a great part in the pathogenesis of radiation sickness when animals are irradiated with the median lethal doses of ionizing radiation and is of no essential significance when they are irradiated with tremendous doses (Warren, Whipple, 1923; Quastler and others, 1952; Gonshery and others, 1953; Bond, 1956, and others).

Above, data were presented on irradiation of sterile (that is, grown under sterile conditions and without body microflora) animals. It was shown that irradiation of them also leads to the development of typical radiation sickness. Under conditions where autoinfection is absent and impossible irradiated animals have only somewhat longer lifespans than the normal. Gonshery and others (1953) determined the existence of autoinfection and the lengths of life of white mice irradiated with x-rays in doses of 550 to 1400 r. It was determined that with

a dose of 1400 r 100 percent of the animals die, whereby in 63 percent of the cases microbes are not found either in the blood or in the spleen. When mice are irradiated with a smaller dose (800 r) the number of animals which die without autoinfection amounts to a total of 20 percent, that is, three times less than in the first case. The dose of radiation of 550 r is lethal to half of the animals, of which only eight percent dies without the presence of microbes in the blood or spleen. The lifespans of such mice are 14.7 days, whereas mice which die with the presence of bacterial seeding of tissues lived only 11 days, on the average. The latter indirectly indicates the fact that endogenous infection which develops from the effect of median lethal doses of radiation aggravates the main pathological process. Convincing facts attesting to the aggravating influence of endogenous infection in radiation sickness have been obtained as the result of experiments on antibiotic therapy of it. Actually, if autoinfection plays the part of an additional pathological factor, antimicrobial therapy should exert a favorable effect. Data in the literature demonstrating this are exceedingly numerous.

In the experiments of Miller and others (1950) the subcutaneous injection of streptomycin 24 hours after irradiation of the mice reduced their mortality rate to 16 percent by comparison with 81 percent in the control. Reduction of the mortality rates of irradiated white mice as the result of treatment with streptomycin was also observed by V. F. Sosova (1954). Furth and others (1952) obtained similar results in the treatment of white rats irradiated with x-rays in a dose of 700 r. Daily oral administration of the antibiotic for 28 days led to a greater survival of treated animals, less loss of weight, reduction of the degree and the frequency of diarrhea. These authors in two other works (1952) described the therapeutic effect of aureomycin, given to dogs orally which had been irradiated in a dose of 450 r. The mortality rate in the control group amounted to 58 percent; in the treated group, 44 percent. The number of positive blood cultures in the group of treated animals was 2.3 times less. However, changes in the hematologic indices in all dogs were the same. This may be considered a fact attesting to the thesis that the favorable effect of antibiotic therapy depends chiefly on its antimicrobial effect rather than on any nonspecific effect. V. L. Troitskiy and M. A. Tumanyan (1955) showed that treatment of irradiated rabbits with antibiotics (combination of levomycetin [levo-rotary chloromycetin], streptomycin and penicillin) either prevents the development of bacteremia or stops it. The mortality rate of the animals thereby is reduced from 90 to 32 percent.

In view of the fact that the main source of infection of the irradiated organism is the intestine, by actions directed at reducing the pathogenic properties of the intestinal microflora a reduction should be achieved in the degree of autoinfection and, by the same token, a favorable influence should be exerted on the course of radiation sickness. Actually, if lactose, which provides for the transformation of the ordinary intestinal microflora into a lactic acid flora, is added to the usual diet, the lifespans of irradiated white rats are increased, the loss of weight is reduced, and the development of diarrhea and areas of necrosis is retarded (R. V. Petrov, 1955; see the description of experiments in Chapter II, section 1).

All these data are evidence to the effect that endogenous infection occurring after irradiation exerts an additional pathological influence aggravating the main process.

Significant along this line are the studies of Bennet and others (1951), made on dogs exposed to a whole body irradiation with x-rays in doses of 350-450 r. Of the 22 dogs which died 17 died after 18 days; the development of endogenous infection was observed in them in all cases. Five of the animals died early, and postmortem blood cultures of them gave negative results. Thereby, reduction of the red blood counts of these dogs was approximately two times less than in the dogs which died with autoinfection. On the basis of the data presented the author draws two conclusions: first of all, infection in radiation sickness aggravates destruction of erythrocytes; secondly, infection plays no considerable part in death from irradiation in the early periods of radiation sickness, subsequently acquiring a leading part along with hemorrhagic phenomena. Essentially, this conclusion is like that of Lawrence and Tennant (1937): "The mechanism of death after irradiation is a combination of tissue destruction and enterogenous infection." The same conclusion is drawn by Tullis and Warren (1947): "Death in the first month after irradiation with lethal amounts of ionizing radiation is apparently the result of three factors acting separately or in combination: anemia, secondary infection and toxemia in combination with tissue degeneration."

These authors are incorrect in the sense that they characterize the thanatogenesis in an exceedingly narrow and one-sided manner after the effect of ionizing radiation on the body. They do not take into consideration the role of functional disorders of organs and systems, particularly of the nervous system (P. D. Gorizontov, 1954; I. A. Pigalev, 1954; A. V. Lebedinskiy, 1955), the role of autosensitization (see part three) and others. However, the formulations pre-

presented above correctly emphasize the important part of endogenous infection in radiation sickness.

All this was stated in the resolution of the discussion on the problem of the mechanism of action of ionizing radiation on the body held by the Central Roentgeno-Radiological Institute of the Ministry of Health USSR and by the chair of medical radiology of the Institute of Advanced Training of Physicians imeni S. M. Kirov: "The role of infection, insignificant in the first stages of radiation sickness, can come out as an accessory factor complicating the course of the main pathological process in the second-third week of the sickness." What has been stated is in complete agreement with the material on the study of those affected by the explosion of atomic bombs in Hiroshima and Nagasaki (Le Roy, 1947; Brues and others, 1947) as well as observations on patients injured from an accident at a uranium reactor (L. Hempelmann, H. Lisco and J. Hoffman, 1952). Specifically during this period infectious complications and septicemia were most frequently observed among those afflicted, and inflammation of the lungs was one of the causes of death. Therefore, speaking of the role of endogenous infection in the pathogenesis of radiation sickness it should be kept in mind that it is of no significance or does not even develop when the organisms are irradiated with doses of ionizing radiation above the lethal dose, when death occurs after three-four days. Conversely, after irradiation with median lethal doses, where the length of life is two-three weeks, endogenous infection inevitably develops and plays the part of an accessory factor complicating the course of the main pathological process. In a number of cases an infectious complication can be the direct cause of death.

Aside from the role of bacteria penetrating into the internal milieu of the irradiated organism as the cause of the autoinfectious complication, they can also be the cause of auto sensitization (P. D. Gorizontov, 1955, 1959). This has not been ruled out theoretically (see the section: "Is Autoimmunization of the Irradiated Organism Possible?"), despite the marked depression of antibody formation. Unfortunately, no experimental study has been made to date. The experiments of V. F. Sosova (1956) exclude the possibility of development of increased sensitivity to the colon bacillus only for the Schwartzmann phenomenon type. Other aspects of this probably important phenomenon have not been studied.

4. Principles of Antibiotic Therapy in Acute Radiation Sickness

In 1955, V. L. Troitskiy and M. A. Tumanyan in experiments on rabbits and, in 1956, M. A. Tumanyan and Z. V. Shevtsova in experiments on monkeys, came to the following conclusions after treating the acute radiation sickness with antibiotics:

1. Broad spectrum antibiotics should be used.
2. The antibiotics are advisably administered in such a way as to create the maximum concentration of them not only in the blood but also in the intestine.
3. Early onset of therapy is needed.

In 1958, we in cooperation with V. D. Rogozkin published the work: "Principles of Antibiotic Therapy in Acute Radiation Sickness" (R. V. Petrov and V. D. Rogozkin, 1958). An analysis of a number of our own data and data in the literature as well as numerous experiments performed by workers in P. D. Gorizontov's laboratory have made it possible for us in this article to confirm the correctness of the principles proposed by V. L. Troitskiy and M. A. Tumanyan and to formulate three more. In this section we shall not present all the experiments which we and V. D. Rogozkin used in the article mentioned but only the main ones. The reservation should also be made that principles of antibiotic therapy are essentially formulated like principles of application of an antiinfectious factor. All the data are regarded from the viewpoint of the microbiologist, and the leading mechanism of action of antibiotics is considered the antimicrobial effect. At the present time, there is very considerable material demonstrating the relativity of such an interpretation (see, for example, the material of the Second All-Union Conference on Antibiotics, 1957; the report of Bilibin to the meeting of the Academy of Medical Sciences USSR, 1960).

Antibiotics as chemical agents possess a pharmacologic effect which is not associated with their antibacterial activity. Unfortunately, this aspect of the action of antibiotics has been far from completely studied, particularly in radiation sickness. Therefore, it is not possible in any exhaustive way to consider this aspect of their action in formulating the principles of antibiotic therapy of radiation sickness. However, this aspect of the action of antibiotics is expediently demonstrated under conditions of radiation injury.

Recently (1960), at an interlaboratory conference organized by N. N. Klemparskaya and V. L. Troitskiy, N. N. Klemparskaya reported on her observations, in cooperation with N. V. Rayeva, on the ef-

effectiveness of antibiotic therapy of acute radiation sickness in dogs. They established the fact that antibiotic therapy of radiation sickness is effective, despite the occurrence of a large number of antibiotic-resistant bacteria as the result of administration of antibiotics. We, in cooperation with A. I. Zhuravlev and V. N. Benevolenskiy (1960), observed the protective effect of antibiotics without antimicrobial activity in radiation injury. Aqueous solutions of aureomycin and penicillin were kept at 50° C for eight days. The bacteriostatic activity of aureomycin was reduced 250 times; that of penicillin, 2,000 times, according to a determination by the method of serial dilutions with a test microbe, *Staphylococcus aureus* No 209. The bacteriostatic concentration of aureomycin reached 2.5 milligrams per cc. In experiments on the protective effect in irradiation of mice the preparation was injected according to a calculation of one milligram per gram. The protective effect of these antibiotics, demonstrable when administered 30-40 minutes before irradiation in experiments on white mice and yeasts, was not reduced. In Tables 14 and 15 the results of the experiments are shown.

In experiments on mice the protective effect was evaluated by the lifespans of the animals after irradiation. In experiments on yeasts, by the survival of the cells. Thereby, in Table 15 the protective effect of cysteine is shown for comparison. In connection with the fact that the antioxidant activity of such heated antibiotics is completely preserved, A. I. Zhuravlev is inclined to explain the protective effect of the antibiotics in radiation injury by their antioxidant properties. This, to be sure, is one of the mechanisms of action of antibiotics as chemical agents.

The data presented, illustrating the fact that the favorable effect of antibiotics in radiation injury may be associated not only with their antimicrobial activity, do not negate their basic antiinfectious significance. The "unarmed nature" of the irradiated organism with respect to infection requires the use of antibacterial preparations. The best therapeutic agent in this respect is constituted by antibiotics.

Numerous data indicate the undoubted therapeutic value of antibiotics in radiation sickness. However, the effectiveness of different preparations is not the same, and in a number of cases is entirely absent. Even the same antibiotic, used by different methods, gives results which are far from equivalent. For the purpose of confirming what has been stated we can quote from the works of Miller and others (1950), Smith and others (1953), P. N. Kiselev and others (1955), V. L. Troitskiy and M. A. Tumanyan (1955), and many others.

Table 14

**Protective Effect of Aureomycin after Intraperitoneal Injection into Mice
40 Minutes after Irradiation in a Dose of 800 4**

| ① Группа мышей | 1-й опыт ② | | 2-й опыт ③ | | 3-й опыт ④ | | Средняя продол- жительность жизни во всех опытах, сутки | ⑧ Стандартная ошибка средней арифметической | I | P |
|---|--------------------------|---|--------------------------|---|--------------------------|---|--|--|------|-------|
| | ⑤ количество мышей | ⑥ средняя про- должитель- ность жизни сутки | ⑤ количество мышей | ⑥ средняя про- должитель- ность жизни сутки | ⑤ количество мышей | ⑥ средняя про- должитель- ность жизни сутки | | | | |
| ⑨ Конт- рольная | 5 | 6,2 | 10 | 6,0 | 19 | 5,0 | 5,4 | 0,36 | 3,93 | 0,001 |
| ⑩ Защищен- ная (20 мг ауреоми- цина) | 9 | 8,1 | 15 | 8,6 | 20 | 6,4 | 7,5 | 0,38 | | |

1. Group of mice; 2. First experiment; 3. Second experiment; 4. Third experiment; 5. No. of mice; 6. Average lifespan, days; 7. Average lifespan for all experiments, days; 8. Standard error of the arithmetic mean; 9. Control; 10. Protected (20 milligrams of aureomycin).

One of the reasons for this situation lies in the fact that under conditions of acute radiation sickness various authors are usually guided by different principles of administration of antibiotics without considering other important conditions of their actions.

First of all, we should emphasize once again that infectious complications of radiation sickness are etiologically extremely heterogeneous. They can be brought about by the colon bacillus, by cocci, by *B. proteus* or by a combination of several species of microorganisms.

This alone indicates the need for using broad spectrum antibiotics or combinations of preparations. Experiments on testing antibiotics with different spectra in radiation sickness have confirmed the correctness of this principle. Streptomycin, for example, has proved

Table 15

The Protective Effect of Normal and Inactivated Penicillin (Judging from the Influence on the Inactivation of Cell Division in *Saccharomyces Cerevisiae* Irradiated with Gamma-Rays in a Dose of 50, 000 r [*Saccharomyces vini* is now usually called *Saccharomyces cerevisiae*])

| № опыта | Неинактивированные клетки, % | | | |
|---------|---|---|---------------------------------|---------------|
| | пенициллин нормальный (10 ⁻⁵ М/мл) | пенициллин инактивированный (10 ⁻⁵ М/мл) | цистеин (10 ⁻⁵ М/мл) | без препарата |
| 1 | 37,9 | — | 35,0 | 5,7 |
| 2 | 49,6 | 52,4 | 43,7 | 13,1 |
| 3 | 33,5 | 37,3 | 27,6 | 11,1 |
| 4 | 64,4 | 89,1 | 16,1 | 7,8 |

1. No. of experiment; 2. Inactivated cells, %; 3. Normal penicillin (10⁻⁵ moles per cc); 4. Inactivated penicillin (10⁻⁵ moles per cc); 5. Cysteine (10⁻⁵ moles per cc); 6. Without the use of a preparation.

to be more effective than penicillin (P. N. Kiselev and others, 1955). In the treatment of acute radiation sickness in rabbits and monkeys V. L. Troitskiy and M. A. Tumanyan (1955) obtained good results by means of combining a number of antibiotics. In experiments on mice and rats, which we performed in conjunction with V. D. Rogozkin (1958), it was determined that penicillin is less effective than streptomycin or biomydin, while the combination of streptomycin and ecmolin [albumin-free fish liver extract with bacteriostatic properties] exerts a better therapeutic effect than biomydin alone. This was demonstrated both by the survival rate and lifespan as well as by the clinical manifestations of radiation sickness.

We should dwell on the problem of expanding the antibacterial spectrum through combination of antibiotics. Not all antibiotic combinations lead to an increase in the therapeutic effect. It has been shown, for example, that prescribing biomydin together with levomycetin in a number of cases led to unfavorable sequelae, the more fre-

quent occurrence of lethargy, reduced alimentary excitability, a bleeding tendency, and reduction of body weight. In other cases the combined use of antibiotics (biomycin and penicillin) did not cause such considerable disorders but was less effective than the administration of a single -- the better -- antibiotic (biomycin) which was in the combination.

Therefore, expansion of the antimicrobial spectrum by means of combining several antibiotics should be conducted with caution in the treatment of radiation sickness, taking into consideration the possibility of side effects. The latter can make it necessary to stop one antibiotic or another. However, under otherwise equal conditions preference should be given to preparations with broader spectra of action.

The problem of the most efficient method of combining antibiotics will be discussed further later. Now, we should like to emphasize that the first principle of antibiotic therapy of acute radiation sickness is the need for administering antibiotics with a broad spectrum of action on microbes.

Above, it has been shown that infectious complications of radiation sickness do not occur immediately after the irradiation but most often from the end of the first to the third week. However, invasion of bacteria from the intestine and respiratory tract into the internal milieu of the organism begins much earlier, with the second day after irradiation (see section 2 of this chapter). Although various periods of time may pass from the time of penetration of the microbes into the tissues until the development of the infectious complication, antimicrobial agents should be prescribed as soon as possible after irradiation. According to the data of a number of investigators (A. K. Gus'kova and G. D. Baysogolov, 1955; N. A. Kurshakov, I. S. Glazunov, 1955, and others), early administration of antibiotics in radiation sickness has completely justified itself. The experiments of V. D. Rogozkin on irradiated rats, performed for the purpose of finding out the time when biomycin can be prescribed most effectively, have confirmed the expediency of early administration of the antibiotic (Table 16). A single administration, on the day of the irradiation, of antibiotics is not effective or not very effective in the majority of cases (Benes and others, 1957).

In the analysis of Table 16 the need not only for early but also prolonged use of antibiotics attracts attention. This situation is confirmed in studies which show the duration of injury to mechanisms of immunity and of the presence of bacteria in the blood and tissues of

Table 16

The Therapeutic Effect of Biomycin as a Function of the Time of Prescribing it (Data of V. D. Rogozkin)

| Животные и доза облучения, р ① | Количество животных ② | Сроки назначения (введения через рот), сутки ③ | Выживаемость к 45-м суткам ④ | Средняя продолжительность жизни, сутки ⑤ |
|-----------------------------------|--------------------------|---|---------------------------------|---|
| Крысы, 650 ⑥ | 40 | С 1 по 20-е ⑧ | 50 | 16,5 |
| | 40 | С 7 по 20-е | 31 | 15,3 |
| | 40 | С 10 по 20-е | 24,5 | 12,3 |
| | 40 | С 1 по 10-е | 25 | 15,4 |
| | 40 | С 1 по 12-е | 35 | 14,7 |
| | 40 | Контроль облучения ⑨ | 25 | 10,4 |
| Собаки, 600 ⑦ | 5 | С 1 по 20-е ⑧ | 1 | 17,5 |
| | 5 | С 6 по 20-е | 0 | 16,4 |
| | 5 | Контроль облучения ⑨ | 0 | 11,2 |

1. Animals and dose of radiation, r; 2. No. of animals; 3. Time of prescribing preparation (orally), days; 4. Survival to 45th day (in percentages for rats and in absolute figures for dogs); 5. Average lifespan, days; 6. Rats (injected with 10 milligrams each twice a day); 7. Dogs (injected with 0.3 milligram four times a day); 8. From the first through the 20th; 9. Irradiation control.

irradiated animals. Thus, Bennet and others, in experiments on dogs irradiated with x-rays in doses of 350-450 r, recorded peremias until the 28th day. V. L. Troitskiy and M. A. Tumanyan found microbial seeding of tissues of irradiated rabbits for 23 days (section 2 of this chapter).

Therefore, the second important principle in efficient administration of antibiotics in acute radiation sickness is early and prolonged administration of them.

Prolonged administration of antibiotics, however, has inherent in it a minimum of two dangers: first of all, the possibility of

accustomation of the bacteria to the antibiotic; secondly, the possibility of occurrence of an undesirable side effect of the preparation on the macroorganism. The need for avoiding this is obvious, particularly if we take into consideration the fact that antibiotic-resistant bacterial strains occur very easily in the irradiated organism (see section I, Chapter II) and that in radiation sickness the prolonged administration of antibiotics in a number of cases leads to the development of unfavorable clinical and hematologic changes. Many years of experience in the treatment of infectious diseases shows that the use of antimicrobial agents, including antibiotics, in courses makes it possible to avoid many undesirable reactions when they are used for a long time. In connection with this, experiments were undertaken on rats and dogs for determining the value of this method of prescribing antibiotics in radiation sickness. It was assumed that the intervals between the courses are fraught with the danger of development of an infectious complication for the irradiated organism, particularly during the period of the second-third week of the sickness and, therefore, during the intervals between courses of one antibiotic another was described. In the experiments of V. D. Rogozkin (see R. V. Petrov and V. D. Rogozkin, 1958) it was determined that the best therapeutic effect from biomyacin is observed when it is administered in courses, with the prescription of another antibiotic, streptomycin, in the intervals. The experiments were performed on rats irradiated with gamma-rays in a dose of 800 r. Among the controls 15.5 percent survived. Among the animals which received biomyacin in a dose of 10 milligrams twice a day from the first through the 20th day after irradiation 40 percent survived. Rats which also received streptomycin continuously in a dose of 400 units twice a day survived in 32.5 percent of the cases. Animals which received the same doses of antibiotics in courses (four days of biomyacin and then four days of streptomycin) survived in 47.5 percent of the cases.

Therefore, the third principle which is useful as a guide for antibiotic therapy of acute radiation sickness is the administration of antibiotics in courses with alternation of preparations.

Since the source of infectious complications in radiation sickness is chiefly the microflora of the intestine and respiratory tract it is advisable to administer antibiotics directly into the cavity abundantly inhabited by autologous flora. This is particularly important if we consider that in the irradiated organism there is interference with the constancy of the microflora. For example, in the microbial contents of the intestine quantitative and qualitative changes occur: an initial

slight reduction in the number of colon bacilli, *B. proteus* and anaerobes is replaced by a marked increase in their number; thereby, there is an increase in the number of microbe strains which possess hemolytic indole- and hydrogen-sulfide-forming properties (see section 1, Chapter II). Therefore, the pathogenic significance of the intestinal microflora increases in radiation sickness. For this reason, effects directed at inhibiting the pathogenic properties of the autologous flora exert a favorable influence on the course of radiation sickness. The transformation of the ordinary intestinal microflora into a lactic acid flora by means of the addition of lactose to the diets of white rats assures a marked reduction in the number of conditionally pathogenic and pathogenic species of bacteria in the intestine and exerts a favorable effect.

Fifty white rats were divided into two equal groups. One group was changed over to a lactose diet four days before irradiation and it was kept on this diet until the end of the experiment (see the methods in section 1, Chapter II). In Table 17 the times of death of irradiated rats are shown. From the Table it is seen that animals which were on the lactose diet lived longer after irradiation than did the controls.

Table 17

Times of Death of Rats on a Lactose Diet and Control White Rats after Irradiation

| ① Диета | ② Доза облучения, r | ③ Количество крыс | ④ Число выживших крыс на данные сутки | | | | | | | |
|-----------------------|------------------------|----------------------|---------------------------------------|----|----|----|----|----|----|----|
| | | | 4 | 5 | 7 | 9 | 12 | 15 | 20 | 21 |
| ⑤ Обычная | 600 | 25 | 25 | 24 | 14 | 6 | 0 | 0 | 0 | 0 |
| ⑥ Лактозная | 600 | 25 | 25 | 25 | 22 | 16 | 4 | 2 | 1 | 0 |

1. Diet; 2. Dose of radiation, r; 3. No. of rats; 4. No. of rats surviving on the given days; 5. Ordinary; 6. Lactose.

In addition, animals on the lactose diet lost less weight than the controls; areas of necrosis on the extremities and diarrhea developed later in them; they refused food later than did the controls. The refusal of food, accompanied by a stoppage of the intake of lactose into the intestine, evidently gradually eliminates the predominance of the lactic acid flora in the intestine. Beginning with this time (fifth-eighth day) the favorable effect of lactose ceases.

An even greater influence on the intestinal microflora is exerted by antibiotics. Thereby, impoverishment of the microflora leads to a reduction of autoinfection in radiation sickness. Thus, for example, Philipson and Laurell (1958) used terramycin and neomycin with the aim of destroying the intestinal flora in mice irradiated with doses of 550-600 r. This brought about a reduction in the number of cultures which could be plated out of the blood and spleen. In irradiated untreated animals positive cultures were found in 94.3 percent of the cases. When antibiotics were prescribed from the fifth through the 10th day bacteria were isolated in 20.2 percent of the cases; when they were prescribed from the first through the 20th day, in only 4.9 percent. V. F. Sosova (1956) showed the expediency of creating therapeutic concentrations of antibiotics in the respiratory tract in radiation sickness by means of an inhalational method of prescribing streptomycin. She also showed (1959) the significance of local administration of antibiotics in inflammatory processes in the skin.

In connection with these data, the creation of therapeutic concentrations of antibiotics not only in the blood but also in the habitats of the bacteria -- pathogens of the autoinfection -- that is, prescribing the antibiotics orally and by inhalation, is sound. It is very important that with these methods of administration the penetration of antibiotics into the blood occurs by the same routes by which the microbes are incorporated. Therefore, a continuous effect of the antimicrobial preparation on the bacteria is assured: in their habitats, along the routes of their incorporation, and in the internal milieu of the body. However, the use of antibiotics orally or by inhalation only is inadequate. The experiments performed in cooperation with V. D. Rogozkin on 30 dogs irradiated with doses of 270-700 r convince us of this. The animals were given preparations (biomycin with streptomycin in courses) by mouth only. It was determined that by this method of prescribing antibiotics it was almost never possible to isolate bacteria of the colon group from the blood of the dogs. The majority of microbes isolated from the blood were representatives of the microflora of the respiratory tract (staphylococci, streptococci, micrococci). In

control animals, in the majority of cases, intestinal microorganisms were plated out (colon bacillus, *Proteus vulgaris*).

Therefore, oral administration of antibiotics considerably reduced autoinfection from the intestine but exerted no pronounced effect on infection with microbes which lived in the respiratory tract. Therefore, under conditions of antibiotic administration orally in a number of cases the parenteral mode of administration of them proved useful also, particularly in the presence of bacteremia caused by a coccal group of microbes. In these cases, additional prescription of penicillin intramuscularly eliminated the bacteremia. The parenteral mode of administration of antibiotics was undoubtedly necessary in the case of persistent vomiting, profuse diarrhea or with persistent temperature elevation. However, captivation with intramuscular, subcutaneous or intravenous injection of the preparations in radiation sickness is fraught with the danger of development of considerable hemorrhagic phenomena. This principle once again causes us to recommend the use of antibiotics orally and in the form of inhalations, by the same token producing therapeutic concentrations of them not only in the blood and tissues of the irradiated organism but also in the natural habitats of the commensal microbes. Parenteral administration of antibiotics should play an auxiliary role.

Therefore, the fourth guiding principle of antibiotic therapy in radiation sickness is the creation of bacteriostatic concentrations of antibiotics not only in the blood and tissues of the body but also in the habitats of the pathogenic bacteria of autoinfections.

A central place in the problem of reason for failures in antibiotic therapy of radiation sickness is occupied by their influence on the macroorganisms. Antibiotics are far from indifferent agents. Under their influence there is a change in the oxidative processes in the tissues (A. V. Gor'kova, 1952; L. Kh. Kechker, 1951), the secretory processes (V. Ya. Shlapoberskiy, 1952), phagocytosis and antibody production can be activated or depressed (Kh. Kh. Planel'yes and N. V. Churnachenko, 1956; N. N. Klemparskaya and others, 1959). At the present time, considerable material has been accumulated attesting to the possible occurrence of an unfavorable side effect on the body with prolonged prescription of antibiotics. Thus, I. A. Kassirskiy and coauthors (1956), A. L. Myasnikov and coauthors (1950), Brown and others (1951) and many others have described the development of allergic reactions with hemorrhagic phenomena. Womack and Reiner (1951) and others have reported on cases of marked depression of hematopoiesis to the point of development of agranulocytosis. Accord-

ing to the data of many investigators (Long and others, 1949, L. O. Gromashevskaya, 1956), after the administration of antibiotics vitamin deficiency develops, particularly of vitamins of the B group, nicotinic acid, P, C, folic and pantothenic acids. The occurrence of vitamin deficiency, in the opinion of many authors, is associated with a number of factors: antibiotics change the intestinal microflora, which plays a great part in vitamin synthesis, interfere with the absorption of vitamins from the intestine, increase their excretion from the body and increase the organism's need for vitamins. The observations of V. D. Rogozkin (see R. V. Petrov and V. D. Rogozkin, 1958) on animals under conditions of radiation sickness have also shown that some antibiotics (levomycetin, streptomycin), particularly in combinations, are capable of increasing the hemorrhagic phenomena and leukopenia. In other words, antibiotics can exert an unfavorable effect on systems which even without this are markedly injured from the effect of ionizing radiation. In connection with this, the problem arises of preventing and eliminating those changes which can develop with antibiotic treatment and which can aggravate the injurious effect of radiation. For preventing this effect of antibiotics, which aggravates the injurious effect of radiation, preparations and combinations of them which possess pronounced side effects (which, for example, cause depression of hematopoiesis, the development of an allergic state, etc.) should be avoided, and drugs should be used which reduce the undesirable effects of antibiotics.

As the result of the experiments of V. D. Rogozkin, N. V. Rayeva, M. N. Fedotova and Ye. N. Shcherbova, it was found that dimedrol [benadryl], citrin, vitamins of the C group, B group and nicotinic acid (Table 18) are such agents. Only the combined use of these preparations with antibiotics has given reliable results in the treatment of radiation sickness.

Therefore, as the fifth principle we can recommend the use of antibiotics in combination with other drugs, primarily with antihistamines, antihemorrhagic preparations and vitamins.

In connection with the marked depressive effect of radiation on immunity and the great part of the microbial factor in the pathogenesis of radiation sickness the usual methods of observing patients are absolutely inadequate. Recently, along with clinical observations and laboratory tests generally used, progressively greater attention is being given to the need for evaluating the immunological status of the irradiated organism (N. N. Klemparskaya, 1956; A. A. Kanarevskaya, 1955).

Our experiments (N. Ye. Yevseyeva, G. T. Ivanenko, R. V.

Petrov, V. A. Razorenova, V. D. Rogoskin and M. F. Sbitneva), performed on more than 100 dogs irradiated with different doses from 170 to 2500 r, showed that when antibiotic therapy is given methodical observation of the state of immunity is of great assistance in treatment. It was found that the state of immunity in many cases determines the course, outcome and is an early sign of deterioration in the condition in radiation sickness. In this respect the most convenient index was the bactericidal activity of the skin, as determined by the N. N. Klemenskaya method. The experiments showed that dogs with a high initial bactericidal power of the skin usually were more resistant to the effect of radiation; they always lived longer and showed less pronounced clinical manifestations of disease than animals which possessed a low bactericidal activity of the skin. Marked deterioration of the bactericidal properties of the skin, occurring suddenly during the course of the sickness, was a precursor of rapid death of the animal. In treatment with antibiotics the bactericidal power of the skin in many dogs was improved even in the case of a considerable depression of it during the course of development of radiation sickness. Deterioration in the bactericidal power was a poor prognostic sign and a signal of inadequate or incorrect prescription of antibiotics. Systematic blood cultures in the treatment of radiation sickness in dogs made it possible to judge not only the presence or absence of bacteremia but also the microbe by which it was caused. In a number of cases, the latter was a decisive argument for stopping one antibiotic and prescribing another or using them in combination. The determination of the sensitivities of the bacteria isolated to the antibiotics used may prove useful; this can be determined by the accelerated method with standard paper discs. The isolation of an antibiotic-resistant strain is also an indication for stopping the antibiotic. Naturally, in the case of local application of the antibiotics for stomatitis, angina, etc. it is advisable to study the microflora of these infectious foci.

Therefore, antibiotic therapy for acute radiation sickness is expediently given, in our opinion, with the following principles as guides: 1) broad spectrum antibiotics should be used; 2) antibiotics should be used early and long; 3) antibiotics should be prescribed in courses, using one antibiotic in the intervals between courses of another; 4) antibiotic therapy should be given against the background of administration of antihistamines, antihemorrhagic preparations and vitamins; 5) bacteriostatic concentrations of the antibiotics should be created not only in the blood and tissues of the irradiated organism but also in the habitats of the microbes -- the pathogens of endogenous

Table 18

Therapeutic Effect of Antibiotics in Combination with Dimedrol [Benadryl], Citrin and Vitamins of the B Group, C, and Nicotinic Acid in Radiation Sickness (Data of V. D. Rogoskin, N. V. Rayeva, M. N. Fedotova, Ye. N. Shcherbova)

| ① Животные и дозы облучения, р | ② Количество животных | ③ Вид лечения | ④ Выживаемость | ⑤ Клинические проявления | | | | | | ⑪ |
|-----------------------------------|--------------------------|--|-------------------|---|--------------|------------------|-----------------|----------------------------------|--|---|
| | | | | ⑥ Средняя продолжительность жизни, сутки | ⑦ Вялость | ⑧ Возбуждение | ⑨ Геморрагия | ⑩ Падение веса на 4% и больше | ⑫ Усиление тошноты, рвоты, диареи после облучения | |
| ⑫ Крысы, 650 | 40 | ⑭ Биоминин по 10 мг два раза в день через рот на 1—4, 8—12, 16—20-е сутки; в перерывах стрептомицин по 400 ед. два раза в день через рот и внутримышечно | 47,5 | 16,4 | 65 | 25 | 67,5 | 72 | 75 | |
| | 40 | ⑮ То же + димедрол по 1 мг, цитрин по 5 мг, витамин С, В ₁ , В ₆ , РР | 49,5 | 17,5 | 60 | 20 | 57,5 | 65 | 65 | |
| ⑬ Собаки, 600 | 40 | ⑯ Контроль | 17,5 | 11,4 | 90 | 75 | 100 | 90 | 85 | |
| | 5 | ⑰ Биоминин по 0,3 г четыре раза в сутки через рот на 1—4, 8—12, 16—20-е сутки; в перерывах стрептомицин по 200 000 ед. два раза в день через рот и внутримышечно | 1 | 17,5 | 5 | 3 | 5 | 5 | 5 | |
| | 5 | ⑱ То же + димедрол по 50 мг два раза в день, цитрин по 40 мг, витамин С, В ₁ , В ₆ , РР | 1 | 19,2 | 4 | 2 | 4 | 3 | 4 | |
| | 5 | ⑲ Контроль | 0 | 10,8 | 5 | 5 | 5 | 5 | 5 | |

[Legend of Table 18 continued on next page]

[Legend of Table 18 from previous page]

1. Animals and dose of radiation, r; 2. No. of animals; 3. Type of treatment; 4. Survival (the figures characterizing the survival and clinical manifestations are expressed in percentages for rats and in absolute figures for dogs); 5. Average lifespan, days; 6. Clinical manifestations (the figures characterizing the survival and clinical manifestations are expressed in percentages for rats and in absolute figures for dogs); 7. Inertia; 8. Diarrhea; 9. Hemorrhages; 10. Weight loss of 4 % or more; 11. Reduction in the white blood count to less than 1,000 per cc; 12. Rats; 13. Dogs; 14. Biomycin in a dose of 10 milligrams twice a day orally on the first-fourth, eighth-12th, 16th-20th days; in the intervals, streptomycin in a dose of 400 units twice a day orally and intramuscularly; 15. The same plus dimedrol in a dose of one milligram, citrin in a dose of five milligrams, vitamins C, B₁, B₆, and PP [pellagra-preventive vitamin or nicotinic acid]; 16. Control; 17. Biomycin in a dose of 0.3 gram four times a day orally on the first-fourth, eighth-12th, 16th-20th days; in the intervals, streptomycin in a dose of 200,000 units twice a day orally and intramuscularly; 18. The same plus dimedrol in a dose of 50 milligrams twice a day, citrin in a dose of 40 milligrams, vitamins C, B₁, B₆, and PP.

infection (in the intestine and respiratory tract); 6) methodical control should be exercised over the state of the immunological reactivity of the organism.

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Chapter IV

Exogenous Infections

1. Characteristics of the Course of Infections

The phenomenon of more or less prolonged increase in the sensitivity of irradiated animals to infection with pathogenic microbes described in the first chapter is the first characteristic of infectious processes under conditions of radiation injury to the organism. As we have seen, it consists of the fact that the infective dose, that is, the number of microbes necessary for the occurrence of infectious disease under these conditions, is reduced; among irradiated animals a large percentage becomes sick, and the disease which occurs terminates in death more rapidly and more often. The latter apparently occurs as the result not only of a more severe but also of a unique course of the infection in the presence of radiation injury; because it is impossible for the infectious process not to show peculiarities under conditions of impairment of immunity and of the majority of other physiological functions under the influence of radiation (P. D. Gorizontov, 1954; V. A. Lebedinskiy, 1955; I. A. Pigalev, 1955).

First of all, we should dwell on the distinctive nature of the inflammatory reactions in radiation sickness, which consist of inhibition of the development and sometimes even complete absence of the cellular component of inflammation. The predominance of a necrotic component and hemorrhages becomes typical of inflammatory foci. This has been shown in a number of published experiments as well as by observations on people injured by ionizing radiation from an atomic bomb explosion (Liebow and others, 1949).

The study of the characteristics of the course of inflammation in acute radiation sickness was begun long ago. In 1938, V. G. Garshin wrote about qualitative changes in the inflammatory reactions under the influence of x-rays. However, this problem was investigated in detail only in recent years. A great contribution was made by N. A. Krayevskiy and his co-workers (V. V. Shikhodyrov, 1957; A. Ye. Ivanov and V. F. Sosova, 1956; V. I. Ponomar'kov, 1959) as well as by V. F. Sosova (1956), P. N. Kiselev and others (1957, 1958), L. Ya. Ebert (1956) and a number of foreign authors (Liebow and others, 1949; Spiers, 1956). V. V. Shikhodyrov studied inflammation in irradiated rats developing in response to subcutaneous injection of celloidin. It was found that after irradiation with a dose of 300 or 600 r an

exudative-hemorrhagic or fibrinous-hemorrhagic inflammation occurs with the suppression of the proliferative component; irradiation with a dose of 900 r gives rise to the development of a necrotic-hemorrhagic inflammation with a complete loss of the proliferative component.

V. I. Ponomar'kov studied the characteristics of the inflammatory reaction of the peritoneum under conditions of acute radiation sickness. The author produced peritonitis by the intraperitoneal injection of 0.5 percent turpentine emulsion. He also established the fact that suppression of the inflammatory reaction after irradiation does not occur immediately. In the first two-three days after irradiation of the rabbits with a dose of 600 r and during the first day in the case of 1,000 r the peritoneal inflammatory reaction is unchanged. By analyzing his own data and data from the literature, V. I. Ponomar'kov comes to the conclusion, very important for us, that the inflammatory process, which with respect to time fits within the limits of the latent period of radiation sickness, does not undergo any visible changes. Here, it is fitting to mention to the reader the materials of the third section of the first chapter, in which an analysis was made of the time of increase of the sensitivity of irradiated animals to infection. There, a similar conclusion was drawn with respect to infectious processes: if the infectious process is eliminated in two-three days, the sensitivity of the organism to the given pathogen is unchanged in the first few days after irradiation; if we are dealing with a long-term infectious process, the increased sensitivity to the pathogen is demonstrated with infection immediately after irradiation. Above, the characteristics of the inflammatory reaction were described in the reproduction of aseptic inflammation under conditions of radiation injury. The same rules and regulations were demonstrated also through the creation of infectious inflammation.

L. Ya. Eberv (1956) made a detailed bacteriological and clinical-morphological study of experimental pneumonia in rabbits and mice occurring against the background of radiation sickness. This study shows that in pneumococcal pneumonia in irradiated rabbits a serous-hemorrhagic inflammation develops instead of a purulent one. In the infection of irradiated mice with Friedlander's bacillus a purulent-hemorrhagic necrotic pneumonia develops, whereas the same infection of intact mice causes the development of only small-focus bronchopneumonia. Studying the reaction of irradiated animals to intradermal infection with some microbes (colon bacillus, staphylococcus, streptococcus and others), V. F. Sosova (1956) found that the same dose of microbes administered to non-irradiated and irradiated animals

causes different reactions: in the former an infiltrate and hyperemia occur at the site of the injection; in the latter, hemorrhages and necrosis with a black color are prominent. Leukocytic infiltration in the latter case is depressed or entirely absent. There is reason to believe that the inflammatory changes in generalized infections are distinguished by the same uniqueness. Unfortunately, we have not encountered any detailed studies of this problem in the literature. There are only separate observations by authors. N. A. Krayevskiy (1954, 1956) repeatedly pointed to this in autoinfectious complications of radiation sickness accompanied by dissemination of microbes in the internal organs. Histological study of tissues shows that islets of bacterial accumulation in them as well as necrotic foci are not surrounded by a zone of inflammation. V. N. Sivertseva (1956), on macroscopic study of mesenteric lymph nodes in mice affected with murine typhus, established the fact that lymph nodes in the irradiated animals do not enlarge, which does occur in non-irradiated animals. The unique nature of the reactions of lymph nodes after irradiation is indicated also by experiments of P. N. Kiselev (1954), who showed their lower fixative power for pathogenic bacteria (Breslau bacillus [*Salmonella breslau*]).

The change in the cellular reactive processes in virus influenza in the respiratory tracts of irradiated white mice has been described by A. A. Smorodintsev (1958). He studied the developmental dynamics of basophilic and acidophilic inclusions in the cells of respiratory epithelium infected after irradiation of mice.

The basophilic bodies in the cytoplasm of the tracheal and bronchial epithelium in virus influenza were described by Harford and Hamlin (1952). They showed that these bodies are virus accumulations (1955). By studying the morphologic processes in experimental influenzal infection, V. Ye. Pigarevskiy (1957) established the fact that the basophilic inclusions were surrounded by a zone of eosinophilic substance. After the separation of these inclusions the cells freed of viruses in this way do not die but rather regenerate. The author concludes that the formation of the acidophilic bodies is not a destructive process but rather a defense reaction of the cell contributing to the walling-off and isolation of the virus. A. A. Smorodintsev confirmed these data and showed that in irradiated animals this cell reaction occurs differently. In the cells a considerably larger number of basophilic inclusions is found, that is, virus colonies. These inclusions are located not only in the apical but also in the basal portions of the cell. The formation of eosinophilic substance around the virus colonies holds 10 times fewer acidophilic bodies than in the non-

irradiated animals. While in the control animals the reactive process described terminates in the sloughing-off of the inclusions and of the apical portions of the cells with subsequent regeneration of them, in irradiated animals degeneration and breakdown of many epithelial cells of the trachea and bronchi develop.

The unique nature of the infectious processes in radiation sickness is shown also through a study of the number of pathogens in the body tissues. After intraperitoneal infection of non-irradiated rats with *Trypanosoma lewisi* (Naiman, 1944) in a quantity of 10,000-15,000 organisms, the number of parasites in the blood at the height of the disease comes to 200,000-700,000 per cubic millimeter. In animals infected after irradiation with a dose of 300-500 r, the number of parasites per cubic millimeter of blood increases to 1,000,000-2,000,000, that is, three-five times more than in the non-irradiated animals. Something similar is observed after infection of irradiated chicks with the plasmodia of avian malaria (Taliaferro and others, 1954). In the experiments described above with the intradermal infection of animals V. F. Sosova (1956, 1957) found thousands and hundreds of thousands of times more microbes per gram of tissue of the inflammatory focus in irradiated than control rabbits.

Murine typhus (V. M. Sivertseva, 1956) and tularemia (A. S. Shevlev, 1958) in irradiated white mice occur with a more pronounced seeding of the internal organs and blood with the pathogens than in the controls. The same is observed in experimental pertussis infection in irradiated white mice: the number of microbes which can be plated out of a certain quantity of lung tissue considerably exceeds the number of pathogens isolated when the lungs of control animals are cultured (B. N. Sofronov, 1956). The number of viruses in the lungs of irradiated mice in influenzal infection exceeds their number in non-irradiated mice by 10 times (A. A. Smorodintsev, 1957). The number of anthrax pathogens in the blood and liver of irradiated mice 24 hours after infection was tens of thousands of times more than in the same tissues of control animals (A. P. Krasil'nikov and N. A. Izraitel', 1959). The accumulation of large quantities of the pathogens in the infectious foci of irradiated animals is associated with their inevitable entrance into the blood. This characteristic has been described in works mentioned above (B. N. Sofronov, V. F. Sosova) as well as in the studies of P. N. Kiselev (1954, 1956), Brooks (1952), V. N. Sivertseva (1955) and others. All the authors mentioned recorded an early dissemination of the focal infection, typical of irradiated animals, even in those cases when under normal conditions it does not occur at

all. Brooks' observations are particularly demonstrative. They illustrate dissemination of wound infection produced by pathogenic beta-hemolytic streptococci. The experiments were performed on dogs irradiated with x-rays in a dose of 100 r. Immediately after the irradiation the dog was burned (20 percent of the body surface), by applying a plate heated to 60° C to the skin. The wound surface which was formed after several days was spontaneously infected, beginning with the third-fourth day, with beta-streptococcal infection. In 30 out of 40 dogs the beta-streptococcus was found in the blood, whereby septicemia in all cases was observed after the pathogen had appeared in the wound. Streptococci in the wound and in the blood were serologically identical. In 10 animals no dissemination of the beta-streptococcal infection occurred, and they survived. In the control group -- a non-irradiated group of dogs -- the beta-streptococcal complication of the wound surface was not associated with the penetration of cocci into the blood. The mortality rate in this group of animals was six times less. Early dissemination of the infection in irradiated animals is observed also in those cases where the spread of the pathogen throughout the entire body is an obligatory pathogenetic component of the infectious disease. More pronounced signs of dissemination are observed in experimental syphilis in irradiated rabbits (V. I. Samtsov, 1958), poliomyelitis in irradiated monkeys (Syverton and others, 1956), tularemia and anthrax infection in irradiated mice (A. S. Shevelev, 1958; A. P. Krasil'nikov and N. A. Izrael', 1959). On infection of mice irradiated with a dose of 342 r with the first anthrax vaccine [Pasteur's more attenuated anthrax culture, with which an animal is inoculated first; the second vaccine is used after two weeks], a large number of pathogens is found in the blood and liver as early as 12 hours after the infection. In control animals dissemination did not occur even after 18 hours; it was recorded only at the end of 24 hours.

The experiments of V. N. Sivertseva (1956) can also be mentioned on the enteral infection of white mice with the Breslau bacillus. After the infection of intact mice bacteria were first found in the mesenteric lymph nodes on the fifth day; in the liver and spleen, on the eighth. In those irradiated with a dose of 470 r two days before infection the pathogen is found in the mesenteric lymph nodes, liver and spleen as early as on the second day. In this work another characteristic of infectious diseases in irradiated animals is demonstrated -- delay in the process of eliminating the pathogen by the organism.

While in control mice pathogens were found in the liver and spleen only until the 15th day, in irradiated mice they were found until the 30th.

Slower elimination of an infectious pathogen -- the streptococcus -- from the bodies of white rats was reported by P. N. Kiselev (1956); of the influenza virus from the bodies of irradiated rats and mice, by A. A. Smorodintsev (1955). N. N. Klemparskaya (1959) observed this after oral infection of irradiated mice with the typhoid pathogen.

In our experiments (R. V. Petrov, 1957) on the infection of white mice and rabbits with the leptospirosis pathogen a longer stay of it in the tissues of irradiated animals was also observed. For example, in control mice the pathogens of leptospirosis were found in the blood and liver for four days after infection, while in the same tissues of irradiated animals they were found until the end of the second week. The fact is very interesting that in rabbits the duration of the leptospiremic period was strictly dependent on antibody production. The longer the delay in the onset of antibody production in irradiated animals the longer the pathogens were found in the blood. Early occurrence and prolongation of the period of viremia was observed in Cynomolgus monkeys irradiated with a dose of 200 r three days before infection with the poliomyelitis virus (Syverton and others, 1956). N. N. Klemparskaya (1958) ascribes great epidemiological significance to the data on accumulation of large numbers of microbes in the bodies of irradiated animals and prolongation of the period of bacteremia and clearance of the pathogens from the body.

Knowing the depressive effect of radiation on antibody production, another characteristic feature of the infectious processes in radiation sickness should be anticipated -- depression of production of specific antibodies. Despite the practical importance of this problem, it has actually not been studied until recently. Antibody production during the infectious process (rather than as the result of immunization) in irradiated animals was first studied by us in 1956, 1957 through the example of leptospirosis. In subsequent years a number of other works appeared describing the depression of antibody production in experimental influenza (A. A. Smorodintsev, 1957; I. A. Kozlova, 1958) and tularemia (A. S. Shevelev, 1958, 1959). V. I. Samtsov (1958) established the fact that irradiation of rabbits sick with syphilis leads to a delay in the development of a positive Wassermann test. These experiments showed that antibody production in irradiated animals during the course of infectious disease is subordinate to the same rules as have been established for immunization: an absence of changes or a slight depression of antibody production when the antigen is administered simultaneously with the irradiation and a marked depression with a prolongation of the inductive phase when

the antigen is administered after one-two days.

In describing the depression of antibody formation in radiation sickness we should say something about the problem of the diagnostic value of determining antibodies in the blood in one infectious disease or another in an irradiated animal. In view of the fact that infection in such an organism can occur without the appearance of immune bodies in the blood, reactions of the Widal type can lose their diagnostic significance under conditions of radiation injury. Depression of the power of an irradiated organism to develop allergic reactions (Crip and others, 1950; V. F. Sosova, 1956; G. M. L'vitsyna, 1958) devalues allergic tests as diagnostic methods under these conditions. G. M. L'vitsyna made a special study of the developmental characteristics of allergy to pathogenic bacteria under conditions of the effect of ionizing radiation. She established the fact that when allergic tests are performed in irradiated animals not only the complete absence of development of the allergic reaction is observed but in certain periods (during the period of most pronounced signs of radiation sickness) the occurrence of nonspecific skin reactions in response to injection of allergens is noted in more than 50 percent of the cases.

Therefore, the diagnostic serological tests and allergic tests under conditions of radiation injury are of very relative significance. Only the isolation of the culture of a specific pathogen can be considered an undoubtedly authentic diagnostic sign of one infectious disease or another in irradiated animals, because many clinical manifestations of infection can also be distorted. A striking example of this is constituted by the temperature reactions in infectious diseases of irradiated animals. It is known that fever is one of the main general signs of many infectious diseases and that radiation injury is frequently accompanied by elevation of body temperature. Thereby, one of the frequent causes of temperature elevation is the development of infection (P. D. Gorizontov, 1954). Therefore, experiments in which an earlier hyperthermia of experimental animals than of controls is recorded are not surprising. Thus, for example, in guinea pigs infected with *Leptospira icterohaemorrhagiae* a temperature elevation to 40° C or higher was observed from the eighth to the 15th day after infection. Under the same conditions, in those infected after irradiation with a dose of 200 r the temperature elevation was observed during the period from the fifth through the eighth day. The animals died on the ninth-12th day and, despite the striking infection (jaundice, leptospir-emia), after eight days the body temperature in them fell to normal or less (R. V. Petrov, 1957).

In the case of infection of skin-muscle wounds in guinea pigs with the pathogen of gas gangrene (R. V. Petrov, 1957) a characteristic feature of the temperature reaction of irradiated animals is also recorded: the absence of prolonged temperature elevation typical of normal guinea pigs. In irradiated animals the infection led to a brief temperature rise, which even on the second day fell below normal. A considerable drop in the body temperature in gas gangrene in irradiated guinea pigs was observed by A. D. Nadshafov (1959). Observation of rabbits infected intradermally with the colon bacillus at different periods after irradiation (V. F. Sosova, 1956) showed that there may be no temperature reaction during the period of overt manifestations of radiation sickness, even with dissemination of the infection. This phenomenon may be explained by the change in the general reactivity of the body in radiation sickness (P. D. Gorizontov, 1955; A. V. Lebedinskiy, 1955; I. A. Pigalev, 1954) as well as a disorder of temperature regulation (P. D. Gorizontov, 1954; N. A. Volokhova, 1956). The latter does not develop immediately after irradiation. The temperature reaction to the infection of pyrogenal [purified protein-free pyrogenic preparation of bacterial origin] in dogs irradiated with a dose of 400-600 r drops on the third-eleventh day. The injection of a pyrogenic vaccine 24 hours after the effect of ionizing radiation (rabbits, dose 1500-1000 r) causes less of a temperature rise than normal. When the vaccine is injected after 48 hours even a depression of the temperature can be observed, that is, an inverted reaction. Such a change in the reactivity is demonstrated also through study of the leukocyte count in the blood of animals infected after irradiation. It is well known that many infectious processes are accompanied by leukocytosis. In irradiated animals infectious leukocytosis develops only in the early periods after the effect of irradiation, being rapidly replaced by the leukopenia typical of radiation sickness. We observed this in experiments on rabbits infected with leptospirosis at various times after irradiation. The reaction of the intact rabbits to the infection was expressed in the development of leukocytosis which was not very high (15,000-20,000 cells per cubic millimeter of blood) but was prolonged. In those infected after irradiation the leukocyte reaction was different and depended on the time elapsing between irradiation and infection. When infection was carried out on the day of irradiation or 24 hours after it leukocytosis developed on the sixth-eighth day, despite the fact that in the first few days the leukocyte count dropped sharply and was of the "radiation type." Infection performed two days after irradiation caused no increase in the blood leukocytes; a reduction in their number

occurred just as in animals which were radiation controls (R. V. Petrov, 1957).

The absence of leukocytosis in infectious diseases under conditions of radiation injury to the organism has been observed by Brooks and others (1952), Naiman (1944), A. D. Nadzhafov (1959) and other investigators. Ye. A. Dikovenko (1958) made a special study of the effect of ionizing radiation on the course of leukocyte reactions developing in response to injection of milk and cortisone in monkeys and rabbits. In the early periods after irradiation (first-second day) the absence of development of leukocytosis or a paradoxical reaction to the stimulus -- leukopenia -- was demonstrated. During the period from the third to the 10th day after irradiation the injections caused a brief increase in the leukocyte count in the blood by 3,000-5,000. From the 10th to the 25th day paradoxical reactions were again recorded.

Endogenous infectious complications which develop regularly in the "leukopenic" period of radiation sickness do not cause an increase in the white blood count either; the animals die with a profound leukopenia. It is easy to imagine that where both pathological processes -- infectious and radiation -- lead to leukopenia they give a combined effect. The same thing can be observed through the example of experimental gas gangrene in guinea pigs (R. V. Petrov, 1957). After infection of a wound with the gas gangrene pathogen a slight leukopenia is regularly observed (7,000-8,000 leukocytes per cubic millimeter of blood where the normal level is 10,000-11,000). In infected animals after irradiation leukopenia developed more rapidly than in animals subjected to irradiation alone or infection alone and was more severe. Depression of inflammatory and leukocytic reactions, increased multiplication of the pathogens in the tissues, early dissemination of the infection are responsible for the existence of another characteristic feature -- shortening of the incubation period of infectious diseases under conditions of radiation injury. This was demonstrated through the examples of experimental infections: tuberculosis (F. I. Ivanova, 1958), trichophytosis (Ye. A. Karpovich, 1957), syphilis (F. A. Khomich, 1958), and paratyphoid (L. A. Yakovleva, B. A. Lapin and others, 1957).

Therefore, the increased sensitivity of irradiated animals to pathogenic microorganisms -- the pathogens of infectious diseases -- is associated not only with a more severe course of the infection and a higher mortality rate from it but also with the distinctive nature of its manifestations. Thereby, the distinctive features are not the results of a simple summation of the manifestations of two pathological

processes but rather the result of a complex interaction of them. The problem of the mutual influence of radiation injury and infection was posed for the first time pointedly by I. A. Pigalev in 1955 at the First International Conference on Peaceful Uses of Atomic Energy in Geneva. Now it may be stated that this mutual influence may be of two kinds.

First of all, mutual aggravation of both pathological processes. We, in cooperation with V. V. Shikhodyrov (1959), showed this by means of histologic studies through the example of combination of radiation sickness and leptospirosis, where an increase in the pathomorphological changes typical of radiation sickness and leptospirosis was observed. This had been demonstrated previously by O. G. Alekseyeva on the basis of an analysis of clinical manifestations of the combination of radiation sickness and diphtheria infection in guinea pigs (see N. N. Klemparskaya, O. G. Alekseyeva, R. V. Petrov, V. F. Sosova, 1958).

Secondly, a unique "quenching" of one process by the other. Examples of this "quenching" have been described by B. B. Moroz (quoted by I. A. Pigalev, 1955), who showed a delay in the development of tetanus intoxication in irradiated rats; by O. G. Alekseyeva in the study mentioned above. Rigdon and Rudisell (1945) and then Singer (1953) found a low degree of parasitemia under certain experimental conditions in irradiated mice infected with *P. berghei*. At first glance this does not agree with the general rule of accelerated multiplication of microbes in the tissues of irradiated animals.

Singer interprets this unique feature on the basis of two phenomena. First of all, the pronounced tropism of *P. berghei* for young erythrocytes and the absence of their capacity for using mature forms of mouse erythrocytes. Secondly, the rapid disturbance of erythropoiesis as early as 24 hours after irradiation. After comparing this, it is possible to understand the lower level of parasitemia in mice irradiated 24 hours before infection: the cause of it is a smaller number of young forms of red blood cells in the blood of irradiated mice. Stubbs and coauthors (1958) found a similar phenomenon after infection of irradiated mice with trypanosomes (*T. equiperdum*). Since reduction of parasitemia is observed with infection after irradiation this phenomenon cannot be explained by the effect of radiation on the microorganism. On the other hand, parasitemia was reduced only with certain doses of radiation, with 200 r but not with 400 r. Therefore, in this case the mechanism is of a different nature than that in the experiments of Singer. It has not been interpreted but is another illustration of the unusual complicated interaction of two pathological processes.

2. Activation of Latent Infection

The problem of the possibility of activation of latent infection as the result of irradiation, the conversion of chronic into acute forms, the possibility of recurrences of infectious disease after irradiation is exceedingly interesting in a theoretical respect and important in a practical respect. The answer to this question may be of different types for different infections by virtue of the characteristics of their pathogenesis and nature of immunity. It may be supposed that irradiation does not produce activation of a dormant gas gangrene infection, because it does not create conditions necessary for the development of gas gangrene. Necrotic tissues are the basis for the development of the disease, and this cannot be created by irradiation in itself. This has been confirmed by experiments performed on white rats by us in cooperation with V. D. Rogozkin (1959). The animals were infected intramuscularly with the gas gangrene pathogen (*B. perfringens*) in a nonlethal dose. Five-six hours after the infection, edema, including the major part of the thigh, and crepitation were found at the site of injection of the pathogen. However, after 24 hours the focus of infection was walled off and did not spread. After three-four days it consisted of a small dense area, from which in all cases it was possible to isolate a pure culture of the gas gangrene pathogen. Irradiation of these rats with minimum lethal doses of ionizing radiation exerted no apparent effect on the focus of infection: gas gangrene did not develop.

The activating effect of irradiation on a latent infectious process is illustrated by a number of other examples. The experiments of Schechmeister and Adler (1953), V. L. Troitskiy and M. A. Tumanyan (1955, 1956, 1958) deserve the greatest attention. The first-mentioned authors experimented with the R line of mice, for which pseudotuberculosis infection and its spontaneous occurrence were characteristic. Cases of death from this infection under ordinary conditions ranged from 0.5 to four percent in 12 weeks. If the mice were irradiated with x-rays in a dose of 350 or 250 r, the mortality rate from pseudotuberculosis rose to 40-60 percent in four-10 weeks. In order to find out whether activation of latent infection or spread of tuberculosis as the result of increased susceptibility of the irradiated animals plays a part in this increased mortality rate, the next experiment was performed on them. One hundred and ten irradiated mice were divided into two different groups: the first group was placed in a com-

mon cage; the animals of the second group were placed in isolated glass jars after irradiation, which excluded the possibility of post-radiation infection of them from one another. Despite this, during the first three weeks the mortality rate in both groups was the same; during this period about 20 percent of the mice died of pseudotuberculosis. Only beginning with the fourth week was a difference in the mortality rate noted, which then increased: in the first group it continued to increase and reached 60 percent in the eighth week; in the second group, there were practically no cases of death after the third week. This difference is explained by the spread of pseudotuberculosis among the irradiated animals of the first group, which was impossible in the second. In mice which were isolated the reason for the increase in the mortality rate in the first few weeks after irradiation can only be one -- activation of a latent or subclinical form of it.

V. L. Troitskiy and M. A. Tumanyan showed the activation of latent dysentery infection in monkeys after the effect of x-rays. Along this line, the data of Schmitt and Thierfelder (1954) are interesting; they observed the development of herpes zoster one-73 days after completion of a course of x-ray therapy in patients with neoplastic and other diseases. It is well known that the herpes virus is constantly present in the human body in a latent state and produces disease when the body's defense forces are weakened (L. A. Zil'ber, 1956). In this case x-ray irradiation was the factor activating the dormant infection. Talliaferro and others (1945) produced recurrences of experimental malaria in chicks irradiated 20-27, 39 and 72 days after infection.

Therefore, the effect of ionizing radiation not only increases the sensitivity of the organism to infection with the pathogens of infectious diseases and distorts the course of infectious processes but can activate a number of latent or chronic infections.

3. Effectiveness of Specific Prophylaxis

This section is of interest for two reasons. First of all, it is important to know (particularly for the specialist in infectious diseases) the possibility and effectiveness of specific prophylaxis of infectious diseases in radiation sickness. Secondly, comparison of the presence or absence of resistance in an irradiated organism to an infectious agent with the presence or absence of antibodies makes it possible to draw important conclusions of the role and significance of antibodies in immunity. With this aim in view, the basic data on resistance to

Infection in animals immunized before and after irradiation are presented in this section. Thereby, for convenience the effectivenesses of active and passive immunization are being analyzed separately.

The effectiveness of active immunization has been studied in detail in the laboratories of N. N. Klemparskaya, O. P. Peterson, V. L. Troitskiy and in a number of foreign laboratories. In the monographs of N. N. Klemparskaya, O. G. Alekseyeva, R. V. Petrov, V. F. Sosova (1958), V. L. Troitskiy and M. A. Tumanyan (1958) material obtained before 1957 has been summarized. On the basis of these materials very definite conclusions can be drawn.

1. Irradiation of immunized animals considerably depresses their resistance when they are infected in the first few days after irradiation and leads to a complete suppression of acquired immunity when it is tested by means of infection during the period of the developed clinical picture of acute radiation sickness.

2. Active immunization of irradiated animals gives different results with different immunization plans. The injection of vaccine in the first two-three days after irradiation does not increase resistance to infection which has been reduced as the result of irradiation. Later immunization increases the resistance. The later the immunization is performed the more effective it is.

3. Revaccination of irradiated animals is highly effective if the first vaccination was performed before irradiation.

4. During the acute period of radiation sickness the animals show increased sensitivity to vaccinations. Immunization aggravates the course of radiation sickness and increases the mortality rate of the animals.

In contrast to this, immunization performed several days before irradiation exerts a favorable effect on the course of radiation sickness. The rules and regulations of the effects of microbial antigens on the course of radiation injury have been studied in detail by N. N. Klemparskaya (1957) and a number of other authors (Ainsworth, Chase, 1959). These conclusions were drawn mainly on the basis of materials of experiments on mice immunized with typhoid vaccine. Studies of recent years have produced some new facts which have confirmed and rendered more concise the conclusions stated above as well as which provided material obtained on other models with different infections. Perkins and Markus (1957) studied the effectiveness of immunization of mice before irradiation with respect to subsequent infection of them with a *Klebsiella pneumoniae* aerosol. They showed the preservation of a certain degree of strength of artificial

active immunity after irradiation. Thus, for example, infection four days after irradiation causes the deaths of 72 percent of the non-immunized mice and only 10 percent of those immunized. Infections after six days caused the death of 60 percent of nonimmune animals and 36 percent of immune mice. In this work the significance of the radiation dose was shown: 200 r practically does not eliminate artificially created resistance; 300 r depresses it slightly, and only 400-450 r markedly increases the susceptibility to the pneumonia pathogen, making it greater than the natural level, that is, higher than in the non-immunized irradiated mice.

In another work, the same authors (1957) studied the effectiveness of preliminary immunization on other natural infections in mice -- murine typhus and tularemia. As in the previous work, what is particularly valuable here is the fact that the natural route of infection was used. In this case the pathogen was administered by mouth. Immunization against murine typhus resulted in the survival of 72 percent of the mice on subsequent infection. If these animals were infected four days after irradiation 36 percent survived irradiation with a dose of 300 r; 31 percent, a dose of 350 r; 28 percent, 400 r; all died at 450 r. With infection of non-immunized non-irradiated (natural resistance to this dose of pathogen) animals, 36 percent of them survived.

In experiments with tularemia it was shown that irradiation not only eliminates resistance created artificially by immunization but also reduces it below the natural background level, despite vaccination before irradiation. However, it should be noted, and this is an exceedingly important addition to previously existing data, that resistance of animals immunized before irradiation with respect to postradiation infection with the corresponding pathogen is always greater than the resistance of those irradiated without preliminary immunization.

Paulissen and Schechmeister (1958) irradiated mice which had been triply immunized with a vaccine made of *S. enteritidis* using a dose of 350 r. Intraperitoneal infection with this microbe was carried out one or nine days after irradiation. The following values of the LD_{50} for different groups were obtained: $1.3 \cdot 10^8$ for non-immunized non-irradiated mice; $4.4 \cdot 10^3$ for non-immunized irradiated mice; $4.0 \cdot 10^5$ for immunized irradiated mice and $1.0 \cdot 10^6$ for immunized non-irradiated mice. Therefore, irradiation reduces the resistance of immunized animals to the level of non-immunized non-irradiated animals but it, nevertheless, remains higher than in those irradiated without preliminary immunization. R. I. Danilova and coauthors (1959) have come to the same conclusion through the example of tuberculosis

Infection in irradiated guinea pigs. I. Z. Kozlova (1958) concluded the same through the example of influenzal infection in irradiated white mice; I. A. Shabarov (1957), through the example of typhoid infection in mice; Z. V. Shevtsova (1960), through the example of brucellosis infection in guinea pigs.

In our own experiment white mice were vaccinated with typhoid vaccine 21 days before irradiation with a dose of 367 r of gamma-rays. Two days after irradiation the animals were infected intraperitoneally with one or two minimum lethal doses (MLD) (100, 000, 000 or 200, 000, 000 microbes) of the typhoid pathogen (strain No 4446). In Table 19 the results of the experiment are shown.

Table 19

The Effect of Irradiation on the Strength of Active Immunity to the Typhoid Pathogen in White Mice

| ① Внутриопытные группы | ② Количество мышей | ③ Доза облуче- ния, р | ④ Доза воз- будителя, млн, мик- робных тел | ⑤ Пало- маний в течение пяти су- ток после зараже- ния | ⑥ Выжи- вшие животные, % |
|--|--------------------------|--------------------------------|---|---|--------------------------------------|
| ① Контроль облучения | 25 | 367 | — | 0 | 100 |
| ② Облучение + заражение 1 ДЛМ | 25 | 367 | 100 | 25 | 0 |
| ③ Вакцинация + облуче- ние + заражение 1 ДЛМ | 25 | 367 | 100 | 17 | 32 |
| ⑩ Вакцинация + облуче- ние + заражение 2 ДЛМ | 25 | 367 | 200 | 25 | 0 |
| ⑪ Вакцинация + заражение 1 ДЛМ | 50 | — | 100 | 10 | 80 |
| ⑫ Вакцинация + заражение 2 ДЛМ | 25 | — | 200 | 13 | 48 |
| ⑬ Заражение 1 ДЛМ | 50 | — | 100 | 46 | 8 |
| ⑭ Заражение 2 ДЛМ | 25 | — | 200 | 25 | 0 |

1. Groups used in the experiment; 2. No. of mice; 3. Dose of radiation, r; 4. Dose of the pathogen in millions of microbes; 5. No. of mice which died in the five days after infection; 6. Animals which survived, %; 7. Irradiation control; 8. Irradiation plus infection with 1 MLD; 9. Vaccination plus irradiation plus infection with 1 MLD; 10. Vaccination plus irradiation plus infection with 2 MLD; 11. Vaccination plus infection with 1 MLD; 12. Vaccination plus infection with 2 MLD; 13. Infection with 1 MLD; 14. Infection with 2 MLD.

Irradiation considerably reduced the strength of acquired immunity -- not a single irradiated mouse survived infection with two MLD of the microbe. However, after infection with one MLD 30 percent of the animals did survive, whereas all the irradiated mice which had not been immunized died. Very interesting observations were made by G. N. Krivenkov (1960). He showed a marked depression of immunogenesis with respect to the brucellosis pathogen under the influence of irradiation, despite the utilization of a highly effective aerogenic method of immunization. As far as antitoxic immunity is concerned, the strength of it created before irradiation is to a considerable degree preserved after irradiation. Only immunization after irradiation fails to assure the development of subsequent resistance to toxins (Hale, Richard, 1955; Silverman, Chin, 1955; I. M. Goncharenko, 1957; D. R. Kaulen, 1956, 1957; M. A. Shabarov, 1957). The results of study of antitoxic active immunity in irradiated animals also fit within the framework of the next conclusion, which should be added to those previously presented for characterizing the effect of radiation on active immunity: active immunity created before irradiation assures a somewhat increased resistance of irradiated animals to the corresponding pathogen by comparison with irradiated non-immunized animals.

Apparently, the opposite conclusion, drawn previously by M. A. Tumanyan and A. V. Izvekova (1956), is justified only for those specific experimental conditions under which it was derived by these authors.

Passive immunization of irradiated animals and its effectiveness are very important for the prophylaxis of a number of infectious diseases against the background of radiation sickness. The quite high degree of effectiveness of passive immunization in irradiated animals has been described long ago (Naiman, 1944; Hollingsworth, 1950; Adler, Schechmeister, 1952).

Hollingsworth showed that the quantity of passively transferred homologous and heterologous antibodies circulating in the blood of irradiated and control rabbits is the same. Afterwards, this was confirmed and studied in detail by D. R. Kaulen (1956). Thereby, D. R. Kaulen simultaneously determined the level of injected diphtheria antitoxin in the blood of guinea pigs and their resistance to the corresponding toxin. With the same antitoxin titer in the blood of irradiated and control animals the former were many times more sensitive to the toxin. Passive immunization of control animals, which provides for resistance to 30 MLD of the toxin, proves to be ineffective for 90-60 percent of the irradiated animals.

Of recent data in the literature those of Perkins and Markus (1954)

are interesting. They immunized irradiated mice passively 48 hours before aerogenic infection with *K. pneumoniae*. This prevented death of 40 percent of the animals irradiated with a dose of 400 r and 50 percent of those irradiated with a dose of 350 r. Without immunization 100, or 80 percent of the mice died.

Hale and Stoner (1958) showed the reproducibility of a passively transferred state of anaphylaxis in irradiated animals. Ye. V. Karpova (1957) continued her previously published studies, in which she in cooperation with P. N. Kiselev (1956) showed the preservation of a pronounced passive immunity to the *B. perfringens* toxin. However, for the purpose of providing the normal effectiveness of antitoxic serum the injection of quantities of it which are two-three times larger is required. In 1957, Ye. V. Karpova checked the effectiveness of passive immunity by infecting irradiated animals with the gas gangrene pathogen rather than with its toxin. On the seventh day after irradiation with a dose of 450 r the mice were infected, and three hours after this they were injected with antitoxic serum in different quantities. It was determined that during this period of radiation sickness five times more serum is required for the prophylaxis of gas gangrene.

Our experiments (R. V. Petrov, 1957), described in detail in section 4 of the present chapter, also showed the high degree of effectiveness of passive immunization of irradiated animals against such infectious diseases as gas gangrene and tetanus. In contrast to the experiments of Ye. V. Karpova, the animals were infected with the pathogens during the first 24 hours after irradiation. Antitoxic sera were injected after the infection. It was determined that the effectiveness of passive immunity to gas gangrene was the same as normal. The effectiveness of passive immunity against tetanus was reduced, and for the purpose of producing the normal effect the injection of three times the serum doses was required. This difference is associated with the fact that experimental tetanus infection has a longer course than gas gangrene and includes the period of the developed clinical picture of radiation sickness, when sensitivity to toxins is particularly increased (Adler and Schechmeister, 1952; P. N. Kiselev and Ye. V. Karpova, 1956), and the effectiveness of passive immunization becomes less.

Speaking about the relatively high degree of effectiveness of passive immunity in irradiated animals, it should be emphasized that this applies mainly to antitoxic immunity. Above, mention was made of the lower degree of vulnerability of active antitoxic immunity to irradiation by comparison with antibacterial immunity. This applies fully to passive immunity also.

Hale and others (Hale, Stoner, 1954; Hale, Richard, 1955) showed that ionizing radiation does not markedly depress active (this does not apply to vaccinations after irradiation (see the beginning of the section)) or passive immunity to bacterial toxins but considerably depresses the strength of active and passive immunity to bacterial infections. With respect to passive immunity, Hale and Stoner present the following illustration: a mixture of tetanus toxin with tetanus antitoxin injected into irradiated mice does not cause death, but a mixture of pneumococci type III with antipneumococcal serum leads to the death of 94 percent of the irradiated animals, whereas in the control groups death is not observed. Something similar was illustrated by O. G. Alekseyeva (1954). In the experiments of D. R. Kaulen we saw that the presence of specific antibodies in the blood of irradiated guinea pigs assures the survival of part of the animals injected with 30 MLD of the diphtheria toxin. In the experiments of O. G. Alekseyeva the test of strength of immunity in such guinea pigs was made by infection with living diphtheria pathogens. In these experiments, there was no question of 30 MLD; the irradiated animals died of one-two MLD. Thus, in such experiments by numerous investigators antitoxic immunity in irradiated animals is reduced; however, the animals can withstand tens and even hundreds of MLD of the toxin (Silverman, Chin, 1955; I. M. Goncharenko, 1957), do not die of minimum lethal doses of living pathogens (M. A. Tumanyan, A. V. Izvekova, 1956; R. V. Petrov, 1957; N. N. Klemparskaya and others, 1958).

Therefore, the toxin-neutralising power of antibodies in the irradiated organism is preserved and this is sufficient to assure a certain degree of effectiveness of active or passive antitoxic immunity but is absolutely inadequate for preservation of antibacterial immunity, basically cellular immunity.

Taking into consideration the disturbance of the cellular immunity factors in irradiated animals, the lower degree of effectiveness of passive immunity can be explained specifically thereby. Apparently, the state of passive immunity is made up of a minimum of two factors: humoral -- truly passive -- and cellular -- active. The former is assured by direct specific binding of the antigen by antibodies of the blood stream. The second is conditioned by an active cell reaction which has been poorly studied at the present time. It is probably made up of the "assimilation" of passive antibodies by cells, leading to intracellular neutralization of toxins as well as changing the functional capacities of the cells. An example of the latter is increase in the phagocytic activity (opsonisation) of leukocytes after passive immunisation. Contact

[of the cells with the injected antibodies increases their toxin-neutralizing power, which had been depressed after irradiation (P. N. Kiselev and Ye. V. Karpova, 1956). The experimental data presented show that under the influence of irradiation mainly the second factor is injured; the role of antibodies -- the specific fixation of antigen -- is unchanged.

4. Various Experimental Examples Illustrating the Characteristics of Infectious Processes

In the first chapter the results of our own experiments were presented for finding the degree of sensitivity of irradiated animals to infection with the pathogens of leptospirosis, gas gangrene and tetanus. In this section material is being presented of studies of the characteristics of the courses of these infectious diseases under conditions of acute radiation sickness. These three infectious diseases were selected for the purpose of studying the characteristics of the course of infectious diseases, because they represent three different diseases in a pathogenetic respect which occur in a typical form in laboratory animals. They have different immunity mechanisms (antitoxic in gas gangrene and tetanus and antimicrobial in leptospirosis). In addition, study of wound infections is of great interest for military medical practice.

Leptospirosis. The study of leptospirosis in irradiated animals was made in experiments on 27 rabbits, 30 guinea pigs and more than 400 white mice. For the purpose of infecting the animals two strains of leptospiras were used -- *Leptospira icterohaemorrhagiae* and the "Krysa Ramenka" strain. Characteristics of the strains, cultivation methods, methods of infection as well as conditions of irradiation of the animals were described in the first chapter, in which all material illustrating the first characteristic of leptospirosis under conditions of radiation injury -- increased sensitivity of irradiated animals to infection with the leptospiras -- was presented. For the purpose of characterizing the course of the infectious disease after infection we took into consideration not only the mortality of the animals but also the presence or absence of jaundice, made blood cultures and tissue cultures of the liver and kidneys, studied serum antibody titers, weight, body temperature, and the white blood count. The more severe course of leptospirosis in irradiated animals becomes obvious on acquaintance with their mortality rate. For example, after infection of intact mice

with leptospirosis pathogens no deaths are ever observed (see Table 2, Chapter I). Under the same conditions, infection of mice irradiated with a dose of 350-400 r leads to the death of almost all animals. It is curious that with the use of *L. icterohaemorrhagiae* for infection, in irradiated animals intense jaundice develops; in non-irradiated animals, no jaundice develops. Rabbits (see Table 1), like mice, do not die of leptospirosis. If animals irradiated with a dose of 500-600 r are infected, almost all of them die (nine out of 10). Increase in the mortality rate and marked reduction in the lifespan were noted also in experiments on guinea pigs (see Table 3). Below, it is shown that leptospirosis as the cause of death was confirmed by the presence of leptospiremia, the accumulation of specific antibodies in the blood, and by pathological data.

Changes in the body weight were studied in experiments on rabbits infected with a culture of the "Krysa" leptospira and guinea pigs infected with *L. icterohaemorrhagiae*. On comparison of the three experimental groups ("infection control," "irradiation control," and "irradiation plus infection") it was determined that the weight loss was greatest in the third group of animals. For example, rabbits infected 24 hours after irradiation with a dose of 500 r lost 380-590 grams by the 10th day; at this time, animals which were simply irradiated or simply infected did not lose weight or else they lost 50-70 grams. Variation in the body temperature in rabbits was inconstant in all groups. In guinea pigs this index gave clear-cut results: in seven out of 10 animals infected after irradiation with a dose of 200 r a temperature elevation to 40° C and higher was observed during the period between the fifth and eighth days after infection. Of 10 guinea pigs which were simply infected the body temperature was normal in six; in the others temperature elevation was observed on the eighth-15th day.

Study of the white blood count was made in experiments on rabbits. In them after the infection a moderate leukocytosis developed. In the case of infection after irradiation the leukocyte reaction was different and depended on the time elapsing between irradiation and infection. In the case of infection two days after irradiation the leukocyte count fell in the same way as in animals which were simply irradiated. With infection on the day of irradiation or 24 hours after it leukocytosis developed on the sixth-seventh day, although in the first few days the leukocyte count decreased sharply (after the "radiation type"). Apparently, with infection in the late periods after irradiation, when the reaction to radiation injury has already developed, the infectious stimulus does

not produce the leukocyte reaction typical of it. Conversely, if infection is performed in the early periods after irradiation the infection leads to the development of a leukocytosis typical of it (Fig 9).

Characteristic of leptospirosis is the presence of a leptospiremic phase which lasts four-five days and subsequent seeding of the organs with a prolonged (up to several months) existence of leptospiras in the kidneys. In connection with this, in rabbits and guinea pigs the agglutinin titer in the blood was determined periodically and blood was taken every day for culture with the aim of finding leptospiras. Mice were killed at various periods after infection (two-seven mice at each time), and cultures were made of their blood, liver and kidney emulsions. The liver and kidney tissues were emulsified by means of a special instrument for sterile homogenization of organs (R. V. Petrov, 1955). The cultures were kept in an incubator no less than a month. In Table 20 the data of study of white mice at different periods after infection are shown; the infection was produced four days after irradiation. From the Table it is seen that after the infection of normal animals leptospiremia was observed for the first four days; after infection of irradiated animals, seven-13 days. In the kidneys of control animals leptospiras were found for the first five days and then during the period between the 38th and 90th day. In irradiated mice the pathogen was found for the first 16 days and then from the 38th through the 190th day. A study after 220 days showed the presence of leptospiras in the kidneys of both groups of animals. It should be noted that we, like other investigators (Ye. N. Gorshanova, 1955), observed a temporary disappearance of leptospiras from all the tissues investigated. However, in the irradiated animals this period was much shorter (from the 17th through the 25th day) than in the controls (from the sixth through the 25th day).

Antibody formation in leptospirosis in irradiated animals has been described in the second chapter. The simultaneous determination of the duration of the leptospiremic phase during the course of the infection was studied in rabbits and guinea pigs. The results (see Table 11) attest to a depression of antibody production in irradiated rabbits. This depression was very slight in the group of animals (No 1, 3, 6 and 10) infected in the first few hours after irradiation with 600 r: the onset of antibody production in irradiated animals was delayed one-two days by comparison with the controls; leptospiras were found in the blood for six-eight days, whereas they were found from three to five days in the control. On infection of rabbits (Nos 39-42) 24 hours after irradiation with a dose of 500 r a marked suppression

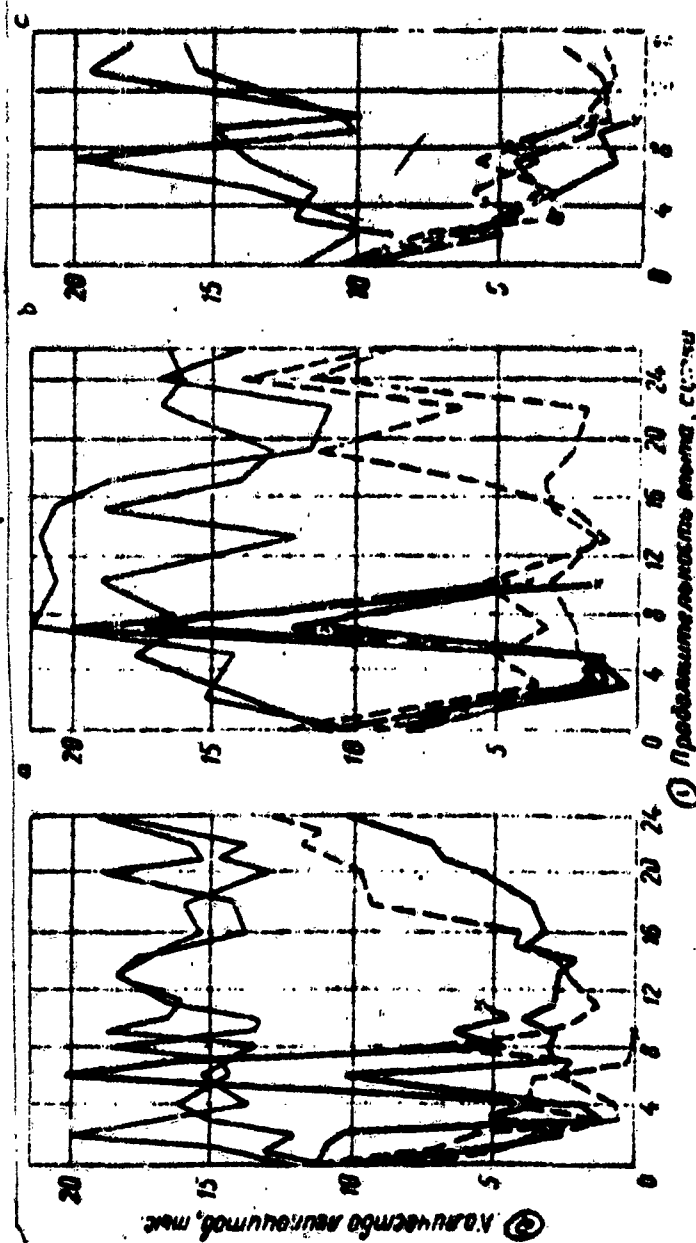


Fig 9. Change in the White Blood Counts of Irradiated and Control Rabbits with Leptospirosis: a. Infection simultaneously with irradiation; b. Infection 24 hrs. after irradiation; c. Infection 2 days after irradiation. Key to the curves: — infection control; --- irradiation control; — irradiation and infection; X - death of the animals. 1. Duration of experiment, days; 2. White blood counts, thousands.

Table 20

Leptospiras in the Tissues of White Mice at Various Periods after Infection

| ① Группа живот- ных | ② Объект иссле- дования | ③ Время после облучения, сутки | | | | | | | | | | | | | | | | | | | | | | |
|--|----------------------------------|-----------------------------------|---|---|---|---|---|---|---|---|----|----|----|----|----|----|-----------|----|----|----|-----|-----|-----|--|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 17- 25 | 38 | 70 | 90 | 141 | 190 | 220 | |
| ④ Конт- роль облуче- ния | ⑤ Кровь Печень Почка | + | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | |
| | | + | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | + | + | + | - | - | + | |
| ⑥ Облуче- ние (350 р) и зара- жение | ⑤ Кровь Печень Почка | + | + | + | + | + | + | + | - | - | - | - | + | + | + | + | - | - | + | + | - | - | - | |
| | | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | + | + | + | + | + | + | |

1. Group of animals; 2. Object of study; 3. Time after irradiation, days (key: +, growth of leptospiras found on culture of the tissue; -, tissue culture negative); 4. Irradiation control; 5. Blood, liver, kidney; 6. Irradiation (350 r) and infection.

of antibody production was observed: the agglutinin titer in the blood of irradiated animals was equal to 1:40-1:1600, with titers in the controls reaching 1:400,000-1:1,600,000; the onset of antibody production was delayed two-three days; leptospiremia lasted nine-10 days. Infection of the rabbits two days after irradiation with a dose of 600 r did not cause the appearance of any antibodies in the blood, and leptospiras were found until the animals died. Experiments on guinea pigs showed the same rules and regulations (see Table 12).

Therefore, we see distinctly that depression and delay of the antibody production process in experimental leptospirosis of irradiated animals are associated with a corresponding prolongation of the period during which leptospiras circulate in the blood. In our opinion, this is important not only as an explanation of the more severe course of leptospirosis in irradiated animals but also as an indication of the important part played by specific antibodies in the pathogenesis of leptospirosis and, particularly, in the mechanism of clearing the leptospiras

from the body. Morphologic changes in experimental leptospirosis in irradiated animals have been studied by us in cooperation with V. V. Shikhodyrov (R. V. Petrov and V. V. Shikhodyrov, 1959) in experiments on guinea pigs. For the infection a culture of *L. icterohaemorrhagiae* was used which had been passaged for a long time on synthetic nutrient media, as the result of which the virulence of the leptospiras had been reduced, and the experimental leptospirosis in the irradiated animals had a subacute course with a low and late mortality and a mild degree of pathological changes (see below). This was extremely desirable, because it afforded a greater possibility for morphological observation of aggravation of the infectious process under the influence of radiation sickness.

In the first chapter (see Table 3) the results of the experiment on three groups of animals were presented: those simply irradiated, those simply infected and those infected on the day of irradiation with a dose of 200 r. A morphological study was made of guinea pigs of all three groups which died or were sacrificed at times corresponding to the times of death of animals in the third group. For histologic examination pieces of the tissues were fixed in 10 percent neutral formalin. Sections of the organs prepared on a freezing microtome were stained with hematoxylin-eosin, picrofuchsin and Sudan. In animals which were radiation controls and which died on the 10th-12th day, on autopsy changes were found characteristic of acute radiation sickness. The hemorrhagic syndrome was expressed quite distinctly. Hemorrhages were observed in the subcutaneous tissue of the trunk; they accumulated chiefly in the area of the back. Multiple large focal hemorrhages were located in the corpus of the stomach, including not only the mucosa and submucosa but frequently also the serosa. In one case the presence of multiple small focal hemorrhages was observed along the course of the small intestine. In the animals of this group the occurrence of hemorrhages in the lungs and muscles of the hind extremities was also noted. The serous membranes of the pleural cavities, pericardium and peritoneum, as well as the subcutaneous tissue of the trunk, were dry. Quite severe changes occurred in the hematopoietic organs. The bone marrow became bright red and liquid. On microscopic examination a reduction in the number of cellular elements of the red and white blood series was noted. The spleen and lymph nodes were reduced in size; on section, the lymphatic follicles were indistinguishable. On microscopic study of these organs complete disappearance of lymphocytes was noted; the follicles consisted of an edematous reticular stroma. The splenic pulp was filled with erythro-

cytes. The lymphatic sinuses were dilated, filled with sloughed-off cells and red blood cells. In the liver, kidneys and myocardium pronounced signs of cloudy swelling were found on microscopic examination. The testicles were reduced in size; in the lumina of the seminiferous tubules there was an absence of epithelium. Therefore, in animals of this group acute radiation sickness developed with the characteristic symptom complex of morphologic changes in the form of occurrences of hemorrhages in various organs, atrophy of lymphoid tissue and aplasia of the bone marrow, degenerative changes in the parenchymatous organs and atrophy of the seminiferous epithelium.

In the second group there were animals which served as infection controls and, therefore, illustrated the morphologic changes in experimental leptospirosis.

Nine-10 days after the injection of leptospiras no gross macroscopic changes could be found, with the exception of a slight enlargement of the spleen. Hemorrhages were absent; a slight icterus was found in one guinea pig. On microscopic study of the organs the considerable increase in the number of Kupffer cells in the liver attracted attention; these had quite intensely stained nuclei and cytoplasm. The liver cells were in a state of cloudy swelling. In places, the cellular elements underwent lysis. The walls of the blood vessels were homogenized, and the connective tissue surrounding them was edematous and, in places, contained fibrin. In a number of organs (heart, lungs, liver) hypertrophy of the vascular endothelium was noted. The lymphatic apparatus of the spleen was considerably enlarged. In the heart and kidneys slight degenerative changes were noted. In animals which died of leptospirosis on the 19th, 42nd and 60th day, scattered hemorrhages were noted in the skin, subcutaneous tissue and diaphragm at autopsy. There was no icterus. The presence of leptospirosis, as in the experimental group, was confirmed by the isolation of leptospiras from the blood and kidneys. The quite mild course of the leptospiral infection observed clinically in these animals was not associated with deep-seated morphologic changes. On the ninth-10th day only hypertrophy of the Kupffer cells and lymphatic apparatus, slight degenerative changes in the parenchymatous organs and lysis of part of the liver cells were noted.

In the third experimental group a study was made of the course of acute radiation sickness in combination with leptospirosis. In the pathological picture changes were observed which occurred as the result of the development of acute radiation sickness as well as because of infectious disease. In animals of this series the hemorrhagic syn-

syndrome was exceedingly marked. An abundance of hemorrhages was observed in all the cases studied. In the subcutaneous tissue of the trunk as well as of the extremities the hemorrhages were of the nature of extensive areas of ecchymosis. Multiple large focal hemorrhages were observed in both lobes of the lungs, in the pericardium, in the depths of the heart muscle. Hemorrhages were also located along the course of the gastrointestinal mucosa: in the mucosa and submucosa of the stomach, duodenum, jejunum, ileum and large intestine. In the majority of animals hemorrhages of extensive size were observed in various muscles, like, for example, in the diaphragm, muscles of the abdominal wall, thigh muscles, back muscles, masticatory muscles and others. Hemorrhages were frequently found in the connective-tissue segments of the retroperitoneal tissue. In various cases the presence of liquid blood in the abdominal cavity was observed. In all animals pronounced icterus of the mucous membranes was noted, and particularly there was intense icterus of the connective-tissue structures (subcutaneous and retroperitoneal tissues). In the hematopoietic organs changes were observed typical of radiation sickness. The liver was somewhat enlarged and of a yellow-gray color. Microscopically, congestion and edema were seen. The liver cells were swollen with homogenized cytoplasm; in places, the cellular elements underwent lysis, forming small areas of necrotic tissue. The number of Kupffer cells was reduced. In the kidneys degenerative changes were seen distinctly, and there was a small number of protein masses in the tubule lumina. The seminiferous epithelium underwent atrophy, and was absent from the majority of tubules. Therefore, in cases of combination of acute radiation sickness and leptospirosis more pronounced changes were observed, typical of both conditions. In such animals there was an intense hemorrhagic syndrome, atrophy of the lymphoid apparatus, of the spleen, lymph nodes, bone marrow aplasia, atrophy of the seminiferous epithelium, icterus of the subcutaneous and retroperitoneal tissues and mucous membranes, the presence of alterative processes in the liver and degenerative changes in the parenchymatous organs. Of the changes typical of acute radiation sickness a more pronounced hemorrhagic tendency in all the tissues than in the control irradiated animals was observed distinctly. In the picture of leptospiral infection there was an increase in the degenerative changes in the liver (areas of necrosis), in the kidneys (the appearance of masses of albumin in the tubule lumina) and other organs. Icterus was expressed more intensely than in the control infected animals. Proliferative

phenomena characteristic of this disease were absent. The number of lymphocytes, Kupffer cells and macrophages was considerably reduced. All the alternative changes under these experimental conditions were expressed to a greater degree than in any cases of infection taken separately. Therefore, after irradiation of rabbits, guinea pigs and white mice with sublethal doses of x-rays the sensitivity of the animals to infection with leptospirosis pathogens increases. The infection, which occurs in a latent form in normal rabbits and mice and does not end in death, ended fatally in the majority of irradiated animals. Experimental icterohemorrhagic leptospirosis, which caused the death of only part of the guinea pigs, ended fatally in all cases where the irradiated animals were infected. Thereby, in those infected after irradiation a more marked icterohemorrhagic syndrome occurred. In irradiated white mice the infection also led to the development of a typical icterohemorrhagic syndrome. With infection of normal mice no icterus developed. Study of the weight and body temperature also showed the more severe course of the infection. The disease occurred without leukocytosis.

In animals infected with leptospirosis pathogens two, 24 and 48 hours after irradiation, antibody production was suppressed; the inductive phase of antibody production was prolonged. The duration of leptospiremia was increased the more the greater the depression in antibody production. The duration of time for which leptospiras were found in the organs of mice infected after irradiation was greater than in the control animals which were simply infected. Histologic studies showed that in animals infected after irradiation a more intense development of pathological signs of both leptospirosis and acute radiation sickness is observed.

Gas Gangrene. Study of gas gangrene in irradiated guinea pigs was made along the three following lines: characteristics of the course of the infection, seroprophylaxis and specific therapy of it under conditions of radiation injury. Animals were infected intramuscularly in the thigh with a culture of *B. perfringens* in a mixture with calcium chloride (a detailed description of the method and irradiation conditions were given in the first chapter); in all the infected animals gas gangrene infection developed as early as after three-four hours. In irradiated animals faster rates of development of the local changes were noted. With the method of infection of normal guinea pigs used, all the signs of gas gangrene infection were present as early as after four hours: tender crepitant edema, temperature elevation to 41°C , disturbance of the function of the extremities. After 24 hours, the gas gangrene

edema spread over the entire thigh, and then went to the inguinal area and further. The body temperature remained high. The number of microbes in the focus of infection is evidence of their more rapid multiplication in the tissues of irradiated animals. For the purpose of making a bacterial count the tissue homogenate was streaked on plates with Wilson-Blair agar, the surface of which was poured over with meat infusion agar after this. From Table 21 it is seen that in the infectious focus of the irradiated guinea pigs there is an accumulation of considerably larger numbers of microbes than in the controls. A particularly marked difference -- by several thousands of times -- is observed in the case of infection with a sublethal dose of the pathogen ($25 \cdot 10^6$ microbes by the optical standard). If the certain lethal dose was used for infection ($150 \cdot 10^6$ microbes), an average of 1.7 times more microbes accumulated in the focus in irradiated guinea pigs than in the controls.

Table 21

The Number (In Thousands) of Microbes per Gram of Affected Tissue in Gas Gangrene in Irradiated and Control Guinea Pigs

| ① Группа свиней | ② Через сутки после заражения дозой $25 \cdot 10^6$ микробных тел | | ③ Через 5 ч после заражения дозой $150 \cdot 10^6$ микробных тел | | ④ Через сутки после заражения дозой $150 \cdot 10^6$ микробных тел | |
|--|---|----------------------|--|----------------------|--|----------------------|
| | ② Из вышесказанного из очага (бедро) | ③ Из очага клетчатка | ③ Из вышесказанного из очага (бедро) | ④ Из очага клетчатка | ④ Из вышесказанного из очага (бедро) | ⑤ Из очага клетчатка |
| ② Необлученные | 63 | 4,9 | 280 000 | 3 | 1 500 000 | 35 000 |
| ③ Облученные дозой 360 р за четверо суток до заражения | 210 000 | 1400 | 490 000 | 51 | 2 700 000 | 33 000 |

Note. Each figure is the arithmetic mean of the results of study of three guinea pigs.

1. Group of guinea pigs; 2. 24 hours after infection with a dose of $25 \cdot 10^6$ microbes; 3. 5 hrs. after infection with a dose of $150 \cdot 10^6$ microbes;
[continued next page]

[continuation of Table 21 from previous page]

4. 24 hours after infection with a dose of $150 \cdot 10^6$ microbes; 5. Muscles from the focus (thigh); 6. Inguinal tissue; 7. Non-irradiated; 8. Irradiated with a dose of 350 r four days before infection.

From the Table it is seen that in irradiated animals there is a more active multiplication of pathogens in the periphery of the focus: five hours after the injection of $150 \cdot 10^6$ bacilli into the thigh muscles the number of microbes in the area of the inguinal tissue was 17 times greater than in the non-irradiated animals. After 24 hours this difference disappeared, apparently as the result of the use of a high infecting dose. Infection with this dose caused the death of all animals at the beginning of the second day. This supposition is confirmed by the fact that when a sublethal dose of the pathogen ($25 \cdot 10^6$) was used for the infection the number of microbes in the inguinal tissues of irradiated animals was several hundreds of times greater than in the controls 24 hours after infection.

After the infection of normal guinea pigs with the gas gangrene pathogen the development of slight leukopenia is constantly observed (7,000-8,000 leukocytes per cubic millimeter). In those infected after irradiation leukopenia was severe and more marked than in animals which were simply irradiated. In Fig 10 changes are shown in the leukocyte counts of irradiated and control guinea pigs infected with the gas gangrene pathogen. In this experiment the infection was produced in the gap of an incised skin-muscle wound 10 hours after irradiation with gamma-rays in a dose of 367 r. With such a procedure the infection has a longer course than after intramuscular infection, and this makes it possible to evaluate in greater detail some of the clinical manifestations of the infection, including changes in the white blood count and body temperature. The typical prolonged temperature elevation for normal guinea pigs in response to infection was absent in those infected after irradiation. In the latter case, infection led to a brief temperature elevation which dropped below normal figures as early as one-two days later.

The characteristics of the course of gas gangrene in irradiated animals described above were confirmed by the experiments of A. D. Nadzhafov (1959) performed on rabbits infected five days after irradiation with a dose of 1000 r. Thereby, he reports some indices which we did not study: a more marked loss of weight, development of anemia

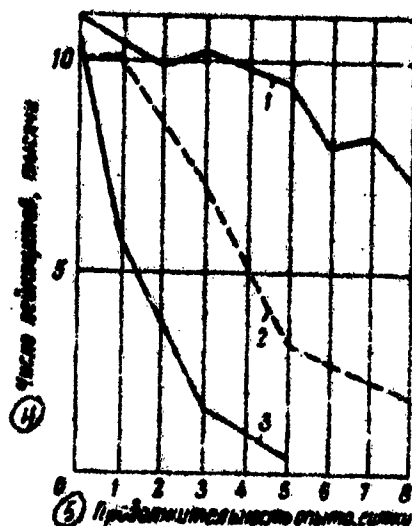


Fig 10. Change in the Leukocyte Count in the Blood of Irradiated and Control Guinea Pigs Infected with the Gas Gangrene Pathogen. 1. Infection control; 2. Irradiation control; 3. Irradiation and infection; 4. White blood count, thousands; 5. Duration of experiment, days.

and early bacteremia in the experimental group of animals.

For the purpose of testing the effectiveness of seroprophylaxis of gas gangrene in irradiated animals guinea pigs were irradiated with gamma-rays in a dose of 367 r 24 hours before infection. Fifteen irradiated and 15 intact guinea pigs were infected with gas gangrene pathogens. Immediately after this, they were injected with antitoxic *E. perfringens* "Diaferm-3" serum. Fifteen uninfected irradiated animals and 10 which were simply irradiated served as controls. The infection was performed in the muscles of the thigh with 0.6 cc of a 1:1 mixture of the microbe culture with a 10 percent calcium chloride solution. The serum was injected into the infection site in a dose of 0.2 cc (160 antitoxic units).

From Fig 11 it is seen that the method of infection used causes the death of 100 percent of the guinea pigs in three-four days. Serum injected into non-irradiated animals prevents the death of the majority of those infected; various animals die beginning with the eighth day.

Injection of serum into irradiated guinea pigs likewise prevents their deaths from gas gangrene: not a single animal died in the first seven days, and then the mortality rate curve coincided with the curve of those which were simply irradiated. This fact, as well as the absence of clinically expressed gas gangrene in the guinea pigs which died, permits us to conclude that the cause of death of the animals was radiation sickness rather than infection. Death of the animals in the group which served as a control for the effect of the serum and in the non-irradiated animals, which was observed after the seventh day, was not caused by gas gangrene (no clinical manifestations of it were observed) but rather by secondary pyogenic infection of areas of the skin which became ulcerated at the infection site.

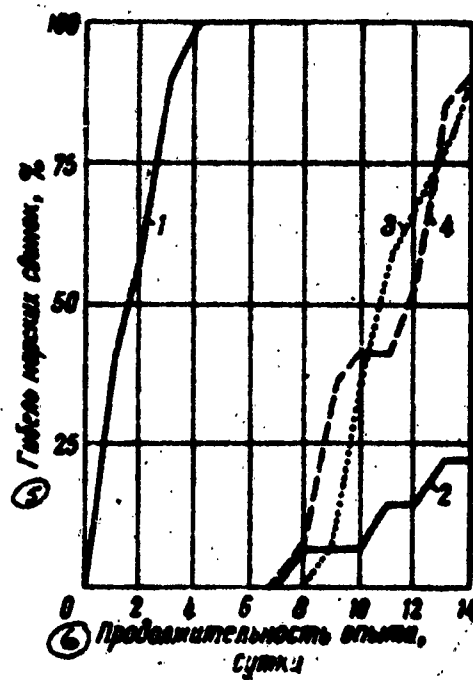


Fig 11. The Effectiveness of Seroprophylaxis of Gas Gangrene in Irradiated Guinea Pigs: 1. Infection control; 2. Control of the serum effect; 3. Irradiation control; 4. Irradiation and infection with administration of serum; 5. Death of normal guinea pigs, %; 6. Duration of experiment, days.

The arrangement and results of the second experiment are shown in Table 22. In this experiment the prophylactic dose of gas gangrene antiserum (200 antitoxic units) was injected 30 minutes after the infection. As the result of prophylaxis none of the experimental guinea pigs died of gas gangrene infection. Moreover, there was not a single case of development of infection in the presence of 100 percent mortality of animals serving as infection controls from gas gangrene.

The second, no less important question is the question of specific therapy of gas gangrene infection in irradiated animals. For the purpose of answering this question several experiments were performed with x-ray and gamma-irradiation in guinea pigs. In experiments with x-ray irradiation the animals received a whole body dose of 200 r each (180 kv, 15 ma, 30-31 r per minute, filters of 0.5 mm Cu plus 1.0 mm Al). Irradiation was performed one-three hours before infection. For the infection the method described above was used which without fail led to the development of gas gangrene with 100 percent mortality. Such infection is necessary so that the effectiveness of the therapy can be evaluated not only from the clinical picture of the infection but also from the survival of guinea pigs. In these experiments a combination of specific antitoxic serum ("Diaferm-3") with penicillin was used for treatment. This combination is most effective according to data in the literature (A. N. L'vov, 1946; Vincent and others, 1947). In our experiments treatment was begun one and three hours after infection. Penicillin in a dose of one-two units per gram of the animal's weight was injected directly into the focus of infection. The serum (0.2-0.4 antitoxic units per gram of weight) was injected into the muscles of the other, the healthy, thigh. In the case of treatment one hour after infection these preparations were injected once. Where therapy was begun three hours after infection the preparations were repeated in the same doses after 24 hours.

The results of the experiments are shown in Tables 23-25. The mortality rate of the animals was evaluated for a five-seven-day period. This is explained by two factors: first of all, in this time usually no cases of death are observed among animals which are simply irradiated (guinea pigs die of radiation sickness after the seventh day). Secondly, death of the animals from gas gangrene occurs in the first three days with the method of infection used. Thereby, the main mass (80-90 percent) dies on the first and second days. In guinea pigs which survive five-seven days as the result of treatment the focus of infection is limited to a zone of infiltration and is encapsulated; at later periods, in these cases death of the animals from gas gangrene is not observed.

Table 22

The Effectiveness of Seroprophylaxis of Gas Gangrene in Irradiated Guinea Pigs

| 1 Доза облуче- ния, p | 2 Содержание опыта | 3 Коли- чество свинков | 4 Продолжительность жизни после заражения, сутки | | | | | | | | | | | |
|---------------------------------|--|---------------------------------|--|---|---|---|---|----|---|----|---|----|----|----|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| 2020 | Облучение + за- ражение + профилактика | 25 | — | — | — | — | 4 | 19 | 2 | — | — | — | — | — |
| | Контроль облу- чения | 10 | — | — | — | — | 2 | 7 | 1 | — | — | — | — | — |
| | Облучение + за- ражение | 10 | 3 | 3 | 2 | 2 | — | — | — | — | — | — | — | — |
| 870 | Облучение + за- ражение + профилактика | 25 | — | — | — | — | 2 | 6 | 6 | 11 | — | — | — | — |
| | Контроль облу- чения | 10 | — | — | — | — | 1 | 3 | 1 | 5 | — | — | — | — |
| | Облучение + за- ражение | 10 | 3 | 2 | 4 | 1 | — | — | — | — | — | — | — | — |
| 570 | Облучение + за- ражение + профилактика | 25 | — | — | — | — | — | — | — | 1 | 5 | 4 | 2 | 4 |
| | Контроль облу- чения | 10 | — | — | — | — | — | — | — | 2 | 2 | 1 | 2 | 1 |
| | Облучение + за- ражение | 10 | 3 | 5 | 2 | — | — | — | — | — | — | — | — | — |
| — | Заражение + профилактика | 25* | — | — | — | — | — | — | — | — | — | — | — | — |
| | Контроль зара- жения | 10 | 2 | 2 | 3 | 2 | — | — | — | — | — | — | — | — |
| 10* Все животные остались живы. | | | | | | | | | | | | | | |

1. Dose of radiation, r; 2. Content of experiment; 3. No. of guinea pigs; 4. Lifespan after infection, days; 5. Irradiation plus infection plus prophylaxis; 6. Irradiation control; 7. Irradiation plus infection; 8. Infection plus prophylaxis; 9. Infection control; 10. All the animals remained alive.

[This is in agreement with data in the literature (A. K. Ageyev, 1954).]

The local manifestations of gas gangrene infection were evaluated on the following scale: G -- accumulation of gas in the tissues; +++ -- edema and infiltrate going beyond the limits of the thigh; ++ -- edema including the whole thigh; + -- infiltrate which did not include the whole thigh; the diameter of it did not exceed one centimeter.

From Table 23 it is seen that the use of gas gangrene antiserum and penicillin one hour after infection of the guinea pigs is effective to a certain degree. Thereby, the fact is interesting that in irradiated animals these preparations used separately are less effective than in non-irradiated animals. Thus, for example, the use of penicillin in animals which are simply infected gives 100 percent survival as against 80 percent survival in the irradiated animals. The same relationship (85 and 60 percent) is observed after treatment with serum alone. Conversely, the use of the combination of both preparations is equally effective in gas gangrene (100 percent survival) of normal and irradiated animals. It is well known that the later therapy is begun for gas gangrene the less the treatment effect. Vincent (1947), for example, presents the following survival rates of dogs treated with penicillin: 100 percent if treatment is begun three hours after infection; 88 percent, if after six hours; zero percent, if after 12 hours. The combination of penicillin with antitoxic serum is effective for 12 hours.

In connection with this, it is interesting to determine the effectiveness of gas gangrene infection therapy in irradiated animals if the combination of penicillin and serum is used later than one hour after the infection.

We performed an experiment in which therapy was begun three hours after infection of the guinea pigs. The results of this experiment are summarized in Table 24. The results in principle are identical with those described above: penicillin alone or serum alone, used for the treatment of gas gangrene in irradiated animals, is less effective than in the controls; the combination of the preparations assures the same survival in both groups of guinea pigs (compare groups 5 and 6 in the Table).

After the need for combined administration of antitoxic serum and antibiotics was established for the treatment of gas gangrene in irradiated animals, two more experiments were performed. In the first, the guinea pigs were irradiated with gamma-rays in a dose of 275 r. They were infected 10 hours after irradiation; treatment was begun four hours after infection. The results are identical (see Table 25).

Table 23

Survival of Guinea Pigs Infected with Gas Gangrene Pathogen (Therapy
One Hour after Infection)

| ① Содержание опыта | ② Количество сви- ном | ③ Местные прояв- ления инфекции через сутки после заражения | ④ Число свином, погибших за семь суток | ⑤ Местные прояв- ления инфекции у выживших живот- ных | ⑥ Число выжив- ших в течение семи суток | ⑦ Местные прояв- ления инфекции к седьмому сут- кам у выживших |
|---|-----------------------------|---|---|---|--|--|
| ⑧ Контроль зараже- ния | 15 | +++Г | 15 | +++Г | 0 | — |
| ⑨ Облучение + за- ражение | 10 | +++Г | 10 | +++Г | 0 | — |
| ⑩ Заражение + сыво- ротка | 20 | +++Г | 3 | +++Г | 17 | ++ |
| ⑪ Облучение + за- ражение + сы- воротка | 10 | +++Г | 3 | +++Г | 6 | ++ |
| ⑫ Заражение + пени- циллин | 10 | +++ | 0 | — | 10 | ++ |
| ⑬ Облучение + за- ражение + пени- циллин | 10 | +++Г | 2 | +++ | 8 | ++ |
| ⑭ Заражение + сы- воротка + пени- циллин | 10 | + | 0 | — | 10 | — |
| ⑮ Облучение + зара- жение + сыво- ротка + пени- циллин | 10 | ++ | 0 | — | 10 | + |
| ⑯ Контроль облуче- ния | 10 | — | 0 | — | 10 | — |

Note. The key is in the text.

1. Content of experiment; 2. No. of guinea pigs; 3. Local manifestations of the infection 24 hours after infection is produced; 4. No. of guinea pigs which died in seven days; 5. Local manifestations of the infection in the animals which died; 6. No. of those surviving for seven days; 7. Local manifestations of infection by the seventh day in those which survived; 8. Infection control; 9. Irradiation plus infection; 10. Infection plus serum; 11. Irradiation plus infection plus serum; 12. Infection plus penicillin; 13. Irradiation plus infection plus penicillin; 14. Infection plus serum plus penicillin; 15. Irradiation plus infection plus serum plus penicillin; 16. Irradiation control; 17. G.

Table 24

**Survival of Guinea Pigs Infected with Gas Gangrene Pathogen (Therapy
Three Hours after Infection)**

| Группы животных №№ | Содержание опыта | Количество сви- ней | Местные прояв- ления инфекции через сутки после заражения | Число опытных погибших за семь суток | Местные прояв- ления инфекции у погибших живот- ных | Число выживших животных через семь суток | Местные прояв- ления инфекции и состояние сви- ней у выживших |
|-----------------------|---|------------------------|--|--|--|--|--|
| 1 | Контроль зараже- ния | 5 | +++Г | 5 | +++Г | 0 | — |
| 2 | Облучение + зара- жение | 5 | +++Г | 5 | +++Г | 0 | — |
| 3 | Заражение + см- воротка | 4 | +++Г | 2 | +++Г | 2 | ++ |
| 4 | Заражение + пе- нициллин | 3 | ++Г | 1 | +++Г | 2 | ++ |
| 5 | Заражение + см- воротка + пени- циллин | 15 | ++Г | 2 | +++Г | 13 | ++ |
| 6 | Облучение + зара- жение + смво- ротка + пени- циллин | 10 | ++Г | 1 | +++Г | 9 | ++ |
| 7 | Контроль облуче- ния | 5 | — | 0 | — | 5 | — |

Note. The key is in the text.

1. Groups of animals; 2. Content of experiment; 3. No. of guinea pigs;
4. Local manifestations of infection 24 hours after infection was pro-
duced; 5. No. of guinea pigs which died in seven days; 6. Local mani-
festations of infection in the animals which died; 7. Number of those
surviving for seven days; 8. Local manifestations of infection by the
seventh day in those which survived; 9. Infection control; 10. Irradia-
tion plus infection; 11. Infection plus serum; 12. Infection plus pen-
icillin; 13. Infection plus serum plus penicillin; 14. Irradiation plus
infection plus serum plus penicillin; 15. Irradiation control; 16. G.

Table 25

Effectiveness of Treatment of Experimental Gas Gangrene in Irradiated Guinea Pigs

| ① Содержание опыта | ② Доза облучения, r | ③ Доза заражающего материала, мл | ④ Доза сыворотки, AE | ⑤ Доза пенициллина, ед. | ⑥ Количество свиней | ⑦ Продолжительность жизни свиней, сутки | | | | | | | | | | | | ⑧ Пало от газовой гангрены, т. е. в течение пяти суток | ⑨ Выжила больше пяти суток |
|--|------------------------|-------------------------------------|-------------------------|----------------------------|------------------------|---|---|---|---|---|---|---|---|---|----|----|----|---|-------------------------------|
| | | | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | | |
| 1. Контроль облучения | 275 | — | — | — | 10 | — | — | — | — | — | — | — | — | 1 | 2 | 1 | — | 0 | 6 |
| 2. Облучение + заражение | 275 | 0.2 | — | — | 10 | 3 | 3 | 2 | 2 | — | — | — | — | — | — | — | — | 10 | 0 |
| 3. Облучение + заражение + лечение сывороткой | 275 | 0.2 | 100 | — | 10 | 2 | 3 | 1 | 1 | — | — | — | — | — | 1 | — | — | 7 | 3 |
| 4. Облучение + заражение + лечение пенициллином | 275 | 0.2 | — | 300 | 10 | 1 | 3 | 3 | 1 | 1 | — | — | — | — | 1 | — | — | 9 | 1 |
| 5. Облучение + заражение + лечение сывороткой и пенициллином | 27 | 0.2 | 100 | 300 | 20 | 2 | 1 | 1 | 2 | — | — | — | — | 1 | 2 | 3 | — | 6 | 14 |
| 6. Заражение + лечение сывороткой | — | 0.2 | 100 | — | 10 | 1 | 3 | — | — | — | — | — | — | — | — | — | — | 4 | 6 |
| 7. Заражение + лечение пенициллином | — | 0.2 | — | 300 | 10 | 1 | 2 | 3 | 1 | — | — | — | — | — | — | — | — | 7 | 3 |
| 8. Заражение + лечение сывороткой и пенициллином | — | 0.2 | 100 | 300 | 20 | 1 | — | 2 | 1 | — | — | — | — | — | — | — | — | 4 | 16 |
| 9. Контроль заражения | — | 0.2 | — | — | 10 | 4 | 3 | — | 1 | — | — | — | — | — | — | — | — | 10 | 0 |

[Reading across] 1. Content of experiment; 2. Dose of radiation, r; 3. Dose of infecting material, cc; 4. Dose of serum, antitoxic units; 5. Dose of penicillin, units; 6. No. of guinea pigs; 7. Lifespan of guinea pigs, days; 8. Died of gas gangrene, that is, in five days; 9. Survived more than five days. [Reading down] 1. Irradiation control; 2. Irradiation plus infection; 3. Irradiation plus infection plus serum treatment; 4. Irradiation plus infection plus penicillin treatment; 5. Irradiation plus infection plus serum and penicillin treatment; 6. Infection plus serum treatment; 7. Infection plus penicillin treatment; 8. Infection plus serum and penicillin treatment; 9. Infection control.

In the last experiment, performed on 170 guinea pigs, the animals were irradiated with different doses -- 570, 870 and 2,020 r. Aside from antiserum and penicillin, biomycin was used. Five hours after irradiation the guinea pigs were infected with a lethal dose of the pathogen of gas gangrene. When the infection developed therapy was begun. In our method of infection the clinically expressed infection was observed as early as after three-four hours (markedly tender crepitant edema, temperature of 41° C or higher, impairment of the function of the extremity, a gas bubble on the x-ray film). Therefore, therapy which we began after four hours was true treatment, which corresponds to the treatment begun with the first clinical signs of gas gangrene in people. Therapeutic preparations were administered every 12 hours for three days. Solutions of antibiotics were injected directly into the focus of the infection; serum, into the healthy thigh muscle. For the first injection penicillin was given according to the calculation 800-1,000 units per kilogram of weight; for subsequent injections, 300-500 units. The dose of serum for the first injection was equal to 300-400 antitoxic units per kilogram of weight; for subsequent injections, 100-200 antitoxic units. The doses of biomycin were given according to the calculation of 300-400 units per kilogram of the animal's weight. Simultaneously with the specific therapeutic preparations the animals were given five cc of isotonic glucose subcutaneously. The glucose was used not only with the aim of detoxication but also as a nutrient substance. This was necessary, because the irradiated animals refused food.

The results of the experiments, summarized in Table 26, permit us to say that such therapy of gas gangrene is effective in animals irradiated with lethal doses. Only two out of 25 guinea pigs infected after irradiation with a dose of 570 r died of this infection. Therefore, therapy is effective in 92 percent of the cases.

Therapy of the infection was approximately of the same degree of effectiveness (in 70 percent of the cases) in guinea pigs irradiated with a dose of 870 r. Only after irradiation with 2020 r does therapy of gas gangrene fail to give the desired results: in all animals the development of infection was observed. However, it was delayed compared with untreated animals.

Thus, specific therapy of gas gangrene with large doses of antibiotics and gas gangrene antiserum is ineffective only when doses of irradiation above the lethal doses are used (2020 r), but this is of no practical interest, because it is impossible to save the animal from radiation sickness after such a dose.

Table 26

Effectiveness of Treatment of Gas Gangrene in Irradiated Guinea Pigs

| 1 Дозы об- лучения, р | 2 Содержание опыта | 3 Колы- чество свинок | 4 Продолжительность жизни жи- вотных после заражения, сутки | | | | | | | | | | | |
|--------------------------------|---|--------------------------------|---|---|---|---|---|---|---|---|---|----|----|----|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| 2020 | 5 Облучение + зараже- ние + лечение | 25 | | 2 | 2 | 6 | 5 | 9 | 1 | | | | | |
| | 6 Контроль облучения | 10 | | | | | 2 | 7 | 1 | | | | | |
| | 7 Облучение + заражение | 10 | 3 | 3 | 2 | 2 | | | | | | | | |
| 870 | 5 Облучение + зараже- ние + лечение | 23 | | | 2 | 5 | 4 | 5 | 5 | 2 | | | | |
| | 6 Контроль облучения | 10 | | | | | 1 | 3 | 1 | 5 | | | | |
| | 7 Облучение + заражение | 10 | 3 | 2 | 4 | 1 | | | | | | | | |
| 570 | 5 Облучение + зараже- ние + лечение | 25 | | | | 2 | | 1 | 4 | 4 | | 3 | 3 | 1 |
| | 6 Контроль облучения | 10 | | | | | | | 1 | 2 | 2 | 1 | 2 | 1 |
| | 7 Облучение + заражение | 10 | 3 | 5 | 2 | | | | | | | | | |
| — | 8 Заражение + лечение | 25 | | | | | | | | | 1 | | | |
| | 9 Контроль заражения | 10 | 2 | 2 | 3 | 2 | | | | | | | | |

1. Doses of radiation, r; 2. Content of experiment; 3. No. of guinea pigs; 4. Lifespan of animals after infection, days; 5. Irradiation plus infection plus treatment; 6. Irradiation control; 7. Irradiation plus infection; 8. Infection plus treatment; 9. Infection control.

With lower radiation doses, such as 570 and 870 r, it is effective. These doses of radiation are lethal. Therefore, with lower, nonlethal doses of irradiation the therapy of gas gangrene should be particularly effective.

Therefore, irradiated animals show increased sensitivity to infection with the gas gangrene pathogen. Thereby, the infection has a more

severe course and has certain characteristic features (accelerated development of gas gangrene edema and multiplication of the pathogen, hypothermia, marked leukopenia and others). Seroprophylaxis of gas gangrene in guinea pigs infected on the first day after irradiation is effective to the same degree as in normal animals. The effectiveness of therapy of experimental gas gangrene is different and depends on a number of factors. First of all, combined administration of antitoxic serum and antimicrobial agents (antibiotics) is obligatory for treatment. The use of serum alone or penicillin alone for therapeutic purposes in irradiated animals is much less effective than in non-irradiated animals. The combination of these preparations is equally effective against gas gangrene in irradiated and non-irradiated animals. (It should be emphasized this this work and, therefore, the conclusions pertain to those cases of gas gangrene where infection with it takes place in the first few hours after irradiation. The effectiveness of therapy will be less if the infection occurs during the period of maximum sensitivity of the irradiated animals to the pathogen (fifth-seventh day). Such data have been obtained by A. D. Nadzhafov (1959). However, of the greatest practical interest is the variant which we have analyzed (for example, in cases of wounds from explosion of an atom bomb)). The significance of comprehensive treatment of infectious complications in radiation sickness has been shown by other authors also (A. P. Krasil'nikov and N. A. Izrael', 1960; B. A. Mokrov, 1960). The experiments of S. Ye. Haryuk (1960) are very interesting. He showed that levomycetin is ineffective for Breslau infection in irradiated mice. If the mice were immunized before irradiation the treatment exerts a favorable effect.

Apparently, the use of antitoxic (serum) or antibacterial (penicillin) agents alone against gas gangrene in the non-irradiated organism is sufficient to eliminate the infectious process. The same treatment, used in the case of gas gangrene in irradiated animals, is less effective. Only the combination of both agents leads to the desired result. Explanation for this phenomenon apparently is the same as the explanation for the facts of greater sensitivity of irradiated animals to infectious agents, that is, depression of leukopoiesis, phagocytic activity, antibody production and others. The data presented indicate the fact that with the occurrence of an infectious disease in an irradiated organism the therapy of the latter should be comprehensive, directed at all essential pathogenetic components of the infection. In addition, the effectiveness of therapy of experimental gas gangrene depends on the time elapsing between infection and the onset of treatment. Thus, with

the use of a combination of serum and penicillin one hour after infection 100 percent of the guinea pigs is cured; such therapy, begun three hours after infection, is effective in 85 percent of the cases. The fact is very important that therapy of gas gangrene of a high degree of effectiveness can be achieved in cases where the treatment is started five hours after infection, that is, when the gas gangrene has already developed. For this, the repeated administration of the combination of antitoxic serum and antibiotics is necessary.

Tetanus. We performed these experiments in cooperation with M. A. Lagun on 300 white mice. The infection was produced intramuscularly with a 24-hour culture of the tetanus pathogen (No 280) grown out on Kitt-Tarozzi medium. The method of infection has been described in the first chapter. A typical picture of ascending tetanus developed in the animals. As early as several hours after the infection the animals limped on the affected extremity. After 24 hours the extremity was absolutely immobile; the tail was rigid; the other hind extremity was not very mobile. On the second-third day the hind extremities and the pelvis were completely paralyzed; the tail was twisted in a corkscrew shape and was immobile; from time to time convulsions of the entire body occurred. Death of the animals began after three days. By the end of the sixth day all the mice had died. This is how tetanus occurred after the infection of intact animals.

Parallel infection of mice 10 hours after irradiation with gamma-rays in a dose of 367 r (dose rate 20.4 r per minute) caused the more precipitous development of tetanus -- all the animals died at the end of the fourth day (see Chapter I, Fig 3).

The test of the effectiveness of seroprophylaxis of tetanus in irradiated animals was made in an experiment on 200 white mice. Fifty animals were infected by the method described above. The second group of 50 mice received the minimum preventive dose of antitoxic antitetanus "Diaferm-3" serum immediately after the infection. The serum was injected intramuscularly in a quantity of 0.1 cc (125 antitoxic units). The third and fourth groups of 25 animals each were infected 10 hours after irradiation and received, respectively, 125 antitoxic units (one dose) and 375 antitoxic units (three doses) of serum.

On Fig 12 the curves characterizing the times of death of experimental animals are shown. From a comparison of the curves it is seen that in irradiated white mice the preventive effect of serum is expressed to a lesser degree than in the non-irradiated animals. Thus, the injection of a single dose of serum (125 antitoxic units) into non-irradiated animals prevents death of 32 percent of the animals from

tetanus. Death occurs during the period from the sixth through the 14th day after infection. The same dose of serum protects only 26 percent of the irradiated mice against tetanus; thereby, they die in earlier periods (five-10 days). The use of triple doses of serum after infection of irradiated animals gives an effect like that which is observed after the administration of a single dose to non-irradiated animals.

Therefore, experiments on seroprophylaxis of tetanus in irradiated white mice showed that its effect is somewhat reduced by comparison with the normal animals, and for the purpose of obtaining the same effect the use of triple doses of serum is necessary.

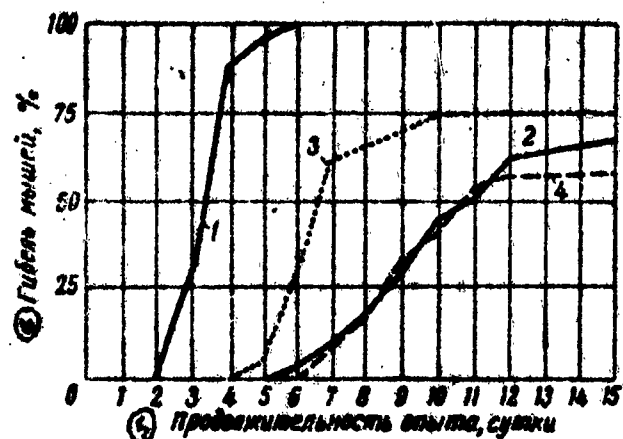


Fig 12. Effectiveness of Seroprophylaxis of Tetanus in Irradiated White Mice. 1. Infection control; 2. Control for the protective effect of a single dose of serum; 3. Irradiation and infection with the use of a single dose of serum; 4. Irradiation and infection with the use of three doses of serum; 5. Duration of experiment, days; 6. Mortality of mice, %.

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THIRD PART

NONINFECTIOUS IMMUNOLOGY OF RADIATION SICKNESS

Introduction

Until quite recently problems of noninfectious immunology included only problems of antigenic differences between animal tissues of different species and problems of incompatibility of blood in transfusions.

The first problem was developed because of the requirements of forensic medicine; the second grew out of the needs of surgery. These two trends contributed a great deal to the development of noninfectious immunology, because they illustrated in a striking manner the significance of this field of knowledge. They were the results of two important discoveries associated with the names of I. I. Mechnikov and Landsteiner. I. I. Mechnikov established the ability of animals to react with antibody production in response to the injection of proteins and associated substances from animals of different species rather than bacterial antigens. His work laid the basis for the study of the species specificity of antigens of the animal organism. Landsteiner was the first to determine antigenic differences between tissues within the species, and discovered blood groups.

The study of antigens of animal tissues has continued to be developed to date. A number of monographs (P. N. Kosyakov, 1954; L. A. Zil'ber, 1958; Wiener, 1943; Landsteiner, 1945; Boyd, 1949) have summarized the results of many years of studies of antigenic differentiation. The existence of other antigens of blood and tissues besides group antigens has been established. M and N antigens, Rh antigens and others have been discovered. They have all been combined into a number of isoantigenic systems (see Dosset's monograph, 1960). The existence of linear antigenic specificity in animals of pure strains has been proved. The existence of functional and organ specificity has been established, that is, antigenic specificity associated with a certain function, with a certain organ, distinguishing this organ from all other body tissues. Recently, the existence of antigenic differentiation has been shown between various cell organoids -- nucleic, mitochondria and microsomes. The existence of organoid specificity is attested to by the data of V. A. Parnes (1957), L. A. Zil'ber (1958), R. V. Petrov and L. I. Il'ina (1959).

L. I. Mechnikov laid the basis for the study of cytotoxins and showed the possibility of production of autocytoxic substances, that is, antibodies against one's own tissues. However, this phenomenon, noted at the beginning of the century, remained little studied for a long time. Only in recent years has the study of autocantigens and autoantibodies become a large-scale study and led to the determination of their important part in the pathogenesis of a number of sicknesses. Therefore, the areas of application of noninfectious immunology have been broadened considerably. Noninfectious immunology has been the clue to the understanding of a number of pathological phenomena.

At the present time, the following divisions of noninfectious immunology can be distinguished: immunology of embryogenesis, immunology of phylogenesis or immunogenetics, immunology of tissue incompatibility in transplantations, immunology of incompatibility between mother and fetus, immunology of cancer, autoimmune diseases of adults, immunology of radiation and burn sickness (N. N. Zhukov-Verezchnikov and R. V. Petrov, 1959). The first three divisions are on the study of normal growth and development processes; the others study the role of immunological mechanisms in the development of pathological processes of noninfectious nature. While the first-mentioned study processes associated with the body's reaction to foreign antigens or to normal developmental antigens, the latter are united by virtue of the fact that they study the occurrence of pathological autoantigens and the body's reaction to them. This branch of immunology has been called "immunopathology" (Grabar, 1960).

In view of the fact that the immunology of radiation sickness studies pathological reactions associated with the occurrence of autoantigens, it is expedient briefly to characterize them.

The three following kinds of autoantigens should be distinguished:

1. Normal developmental antigens, that is, antigenic substances which appear during the course of embryogenesis. Thereby, we have in mind not only the group, organ-specific and other antigens which occur at certain stages of development, previously absent from the embryo, but also particular stage-specific antigens. Stage-specificity of embryonic proteins occurs at certain stages of development, and the antigens responsible for it are specific for these stages only. Description of these antigens and a discussion of their possible part in embryogenesis has been given by O. Ye. Vyazov (1956) and B. V. Konyukhov (1958). Study of these antigens is not included in immunopathology but is the subject of immunology of embryogenesis.

2. Some normal tissues of the adult organism after their entrance

into the blood stream which they do not enter under normal conditions. This has been shown with respect to testicular tissue, tissues of the lens, brain, thyroid gland and others (see the reviews by V. A. Parnes, 1957, 1960; Grabar, 1960). G. V. Lopashov and O. G. Stroyeva (1950) believe that this phenomenon is explained by "the removal" of a number of tissues from the blood stream, in connection with which the proteins of these tissues have not come in contact with the immunological system during the course of embryogenesis and remained "unknown" to it and, therefore, are antigenically active. The correctness of this supposition by G. V. Lopashov and O. G. Stroyeva has been proved by the works of M. Hasek (1953, 1959) and Billingham, Brent and Medawar (1953). They showed that contact between the organism and antigens in the embryonic period assures its tolerance to the given antigen in postnatal life. In connection with this, the suggestion that one's own proteins are not antigens because of the fact that they were in contact with the immunological system during embryogenesis, is very convincing. It also explains the autoantigenicity of tissues "removed from the circulation."

3. Pathologically altered proteins and body substances associated with them. M. G. Sevag (1959) writes that the appearance of such autoantigens can occur as the result of the interaction of normal proteins with such foreign substances as some dyes, drugs, infectious niduses as well as in various injuries. The occurrence of autoantigens as the result of a disorder of protein metabolism can be considered an established fact at the present time (L. A. Zil'ber, 1958; V. A. Parnes, 1961; Claugh, 1960; W. Boyd, 1960).

Autoantigens of the second and third kinds can be the cause of a number of pathological disorders (see the reviews by V. A. Parnes, 1957; Grabar, 1960; M. S. Dul'tsina and Yu. I. Loriye, 1960). At the present time, the important part of autoimmune collisions in the pathogenesis of a number of blood diseases has been proved (J. Dossset, 1960; A. A. Bagdasarov, 1957; A. S. Zverkova, 1957; Miescher, 1953; Finch, 1955), Hashimoto's struma (Roitt and others, 1958; Claugh, 1960), some nephritides (Ye. A. Skal'skaya, 1955), lupus erythematosus (Miescher, 1953; Dameshek, 1956; Z. G. Arlozorov and B. Z. Bron, 1957) and others.

From the viewpoint of noninfectious immunology of radiation sickness autoantigens of the second and third kind are also interesting, because after the effect of ionizing radiation on the body a change occurs in the antigenic properties of tissues and there is increased permeability of histo-hematic barriers providing for the circulation of autoantigens in the blood.

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Chapter V

ANTIGENIC PROPERTIES OF TISSUES OF IRRADIATED ANIMALS

1. Change in the Antigenic Properties of Tissues after Irradiation

The small number of published works which have been accomplished along this line may be divided into two groups. The first proves the change in the antigenic properties of tissues indirectly, by means of the demonstration of antibodies in the blood of irradiated animals against their own proteins. The second group of investigations is on the direct study of tissue antigens after irradiation.

The earliest report about possible change in tissue antigens of the irradiated organism was that of I. P. Mishchenko and M. M. Pomenko (1934). Their idea was based on finding complement-fixing autoantibodies in the blood of irradiated animals. Subsequently, a series of experiments was performed by P. N. Kiselev and his co-workers (1955-1959). The report of the authors mentioned above was confirmed, and this phenomenon was studied in detail. It turned out that after a whole body or local irradiation antibodies appear in the blood of animals against their own denatured proteins. The animal's own serum, denatured by ionizing radiation, alcohol and others, can be used as an antigen for demonstrating these autoantibodies in the complement-fixation test.

In view of the fact that autoantibodies are found for several months, the authors suggest the presence of prolonged denaturation of tissue proteins rather than momentary denaturation in irradiation. In 1956, Bulgarian investigators (Markov and Dimitrov) expressed the idea that there was a change in the antigenic properties of tissues in radiation sickness on the basis of marked erythrophagocytosis observed in the peripheral blood of irradiated rabbits. A Japanese author (Yangisawa, 1959) has come to the same conclusion on the basis of finding auto-precipitins against liver proteins. The experiments were performed in the following way. In rabbits half of the liver area was irradiated with different doses, from 50 to 5,000 r. Autoantibodies were found after irradiation with doses of 500 r or more. Thereby, the higher the radiation dose the higher the precipitin titer and the longer the period they were found in the blood. American investigators (Donaldson, Mitchell, 1959) came to the conclusion that there was a change in the antigenic properties of tissues after irradiation on the basis of experiments of immunization of mice against Ehrlich's carcinoma by means

of transplantation of tumor cells which had been irradiated with a dose of 4,000 r. It was found that immunity to subsequent grafting of the tumor was several times greater than that which occurred after the injection of intact cells. The results of the experiments, in the authors' opinion, can be explained only by a change in the antigenic properties of the irradiated tumor cells leading to an increase in the antigenicity and a reduction of homology. In 1960, N. N. Klemparskaya reported about autoantibodies in the blood of irradiated dogs, guinea pigs and rabbits found by the Høigné method. We, in cooperation with G. M. L'vitsyna (1960), described incomplete autoantibodies in irradiated monkeys, dogs and guinea pigs, demonstrated by the Coombs method.

These are the basic data which indirectly indicate the change in the antigenic properties and the appearance of autoantigenic activity in the body's own proteins after the effect of ionizing radiation on the body.

Direct experiments on the study of antigenic tissue specificity after irradiation were first performed by us and reported at the Leningrad Conference on Problems of the Specificity of Action of Ionizing Radiation on the Body (R. V. Petrov and L. I. Il'ina, 1955). Antigenic differences were established between the tissues of irradiated and normal animals. In subsequent years a number of experiments was performed for the further study of this problem (R. V. Petrov and L. I. Il'ina, 1956-1960; R. V. Petrov, 1957). Independently of us, in 1956 L. A. Zil'ber, V. A. Artamonova, G. M. Frank and A. D. Snezhko published experiments which also indicated a change in the antigenic properties of animal tissues after irradiation. In 1957, Yugoslavian authors (Jankovic, Kanazin and others) described a change in the antigenicity of cell nuclei of the spleens of irradiated rats, and I. N. Mayskiy and G. V. Suvorova (1957) found a change in the antigenic properties of tumor cells after the effect of ionizing radiation on them. In subsequent years (1959) the studies of L. A. Zil'ber and I. N. Mayskiy were continued. S. S. Vasilevskiy, T. A. Fedorova and Ye. M. Belyayeva (1959) published results of immunoelectrophoretic analysis of serum proteins of irradiated rats. By this method differences were recorded in the albumin fraction between the sera of irradiated and control animals.

For the study of antigenic properties of tissues of irradiated animals we used the method of active anaphylaxis with desensitization after L. A. Zil'ber. For this, guinea pigs were sensitized by subcutaneous injection of the preparations; the existence of sensitization was determined by means of administration of the preparations intra-

venously into the hind and fore extremities after 21-30 days. Guinea pigs sensitized with the tissues of normal rats were desensitized with similar substances from irradiated animals and vice versa. After checking on the completeness of desensitization the reacting injection of the antigen used for sensitization was given. The intervals between repeated intravenous infusions were equal to one-two hours. The anaphylactic reaction was evaluated according to the following scale: occasional scratching of the nose and sneezing, $+$; frequently repeated scratching of the nose and sneezing, $+$; the same but more marked and cough, $++$; scratching of the nose, sneezing, cough, involuntary excretion of urine and stool, convulsions, $+++$; death of the animal, $++++$; no reaction, $-$.

By this method various organs and tissues of irradiated rats and rabbits were studied. A study was also made of isolated cell organelles -- nuclei, mitochondria and microsomes of cells.

In making preparations for the sensitization and desensitization of guinea pigs the material was always taken from six-10 animals, and all the procedures were performed on ice or in a special cold room. The homogenates of whole tissues were prepared in the following way. The animals were decapitated. A sample of one organ or another was placed in 12 times its quantity of physiological saline solution, ground up for 10 minutes in a high-speed blender and centrifuged at 3,000 revolutions per minute for 10 minutes. The supernatant fluid was used in the experiment. The cell nuclei were isolated by the Dounce method (1953); the mitochondria and microsomes, by differential centrifugation by the Hogeboom and Schneider method in the G. L. Abelev modification (1956), using Soviet ASL-1 and ASL-2 centrifuges. The preparations were measured out according to the nitrogen content, which was determined by the Kjeldahl micromethod.

In Tables 27-32 the results of experiments are shown which were performed with the tissues of irradiated rats. The irradiation was carried out on a gamma-ray ECO-2 apparatus at a dose rate of 472-341 r per minute. The data for the guinea pigs serving as sensitization controls are not included in the Tables. These animals always reacted to the reacting injection of the antigen with a vigorous anaphylactic reaction, which in various cases terminated in the death of the animal. Guinea pigs serving as toxicity controls for the doses of tissue preparations used have not been included either; injection of the preparation into them caused no apparent reaction. In all the Tables the preparation from the irradiated animals is called the O-preparation; that from the normal animals, H.

For the purpose of evaluating the nature of the change in antigenic properties of one tissue or another, cross desensitization reactions were performed, that is, guinea pigs sensitized with the O-preparation were desensitized with the H-preparation and vice versa. Residual sensitization, demonstrable with the reacting injection of the antigen, attests to the presence of a quality in the sensitizing antigen absent from the antigen used for desensitization. Since the analysis of all the Tables is the same, we shall consider one of them, for example, 27, as an illustration. The upper part of the Table shows the change in the antigenic properties of the liver cell nuclei in radiation sickness in white rats. From the Table it is seen that in guinea pigs sensitized with cell nuclei of non-irradiated white rats no complete desensitization occurs when they are injected with similar preparations from irradiated rats, and guinea pigs show a positive reaction to the reacting injection of the antigen. Conversely, guinea pigs sensitized with the cell nuclei of the same tissues of irradiated animals are completely desensitized by the injection of similar preparations from the normal rats and do not react to the reacting injection of antigens. Therefore, liver cell nuclei and cell nuclei of the small intestinal mucosa taken from irradiated white rats contain fewer antigenic complexes than those of normal animals. The lower portion of the Tables shows the change in the antigenic properties of the liver cell cytoplasm. We see that sensitization created by injection of liver cytoplasm of irradiated rats into guinea pigs is not eliminated completely by the injection of the same preparation from the livers of normal animals. In precisely the same way, sensitization of guinea pigs to liver cell cytoplasm of normal rats is not completely eliminated by injection of cytoplasm from irradiated animals. Therefore, not only is there a reduction in the number of normal antigenic complexes in the liver cell cytoplasm of irradiated rats but simultaneously new substances appear which are not characteristic of the normal cells.

In the composite Table 33 all the results are summarized, and data published by L. A. Zil'ber and coauthors (1956, 1957) are presented on the change in the antigenic properties of some tissues of irradiated rabbits. From this Table it is seen that after irradiation two basic processes develop: the appearance of an antigenic quality not characteristic of the normal and the disappearance of part of the normal antigens; the changes in various tissues are not the same.

Above, it has been pointed out that in all experiments the same investigated tissues from six-10 animals were used for sensitization of guinea pigs. For desensitization and for the reacting injection preparations were also used which consisted of a mixture of the corresponding

Table 27

Antigenic Properties of Liver Cell Nuclei and Cytoplasm Three Days
after Irradiation of Rats with a Dose of 800 r

| ① № животного сыворотки | ② Сенсиби- лизация антигеном | ③ Десенсибилизация | | | | | | | ④ Разрешающее введение | | |
|---|---|---|---|--|-------------------------|---|---|--|---|---|---|
| | | ⑤ Антиген | ⑥ Первое введение | ⑦ Реакция | ⑧ Второе введение | ⑨ Реакция | ⑩ Третье введение | ⑪ Реакция | ⑫ Антиген | ⑬ Доза сыво- ротки, г | ⑭ Реакция |
| 1 2 3 4 5 6 7 | ⑪ Ядра печени нор- мальных крыс | ⑫ Ядра печени облу- ченных крыс | 3 3 3 3 3 3 3 | + ++ ++ ++ ++ ++ + | | 4 4 4 4 4 4 4 | - - ± ± - - - | | ⑪ Ядра печени нор- мальных крыс | 4 4 5 5 4 6 4 | + ++ - ++ ++ ++ ± |
| 8 9 10 11 12 13 14 | ⑬ Ядра печени облу- ченных крыс | ⑪ Ядра печени нор- мальных крыс | 1 2 2.5 3 1.5 2.5 3 | + ++ ++ ++ ++ ++ ++ | | 1.5 2.5 3.5 3.5 2.5 3.5 3.5 | ± - - - - - - | 2 - - - - - - | ⑬ Ядра печени облу- ченных крыс | 6 2.5 5.5 5.5 4 4 4 | - - - - - - - |
| 1 2 3 4 5 6 7 8 9 | ⑬ Цито- плазма печени нор- мальных крыс | ⑭ Цито- плазма печени облу- ченных крыс | 2.5 2.5 2.5 2.5 1.25 1.25 2.5 2.5 2.5 | +++ +++ +++ +++ ++ ++ ++ ++ ++ | | 5 5 5 5 5 2.5 6 6 6 | - - - - - + - - - | 7.5 - - - - - - - | ⑬ Цито- плазма печени нор- мальных крыс | 5 7.5 7.5 7.5 6 7.5 5 2.5 3.5 | +++ ++ ++ ++ ++ ++ ++ ++ ++ |
| 11 12 13 14 15 16 17 | ⑭ Цито- плазма печени облу- ченных крыс | ⑬ Цито- плазма печени нор- мальных крыс | 1.25 1.25 2.5 2.5 2.5 1.25 1.5 | ++ ++ ++ ++ ++ ++ ++ | | 3.5 3.5 5 3.5 3.5 5 3.5 | - - ± - ± + + | 5 - - - 5 - - - | ⑭ Цито- плазма печени облу- ченных крыс | 5 5 10 3.5 7.5 5 7.5 | + ++ ++ + ± ++ ++ |

[Legend for Table 27 on next page]

[Legend for Table 27 from previous page]

*Dose of injected protein for liver cell nuclei, 10 milligrams; for liver cell cytoplasm, 25 milligrams. **Dose of protein in mg.
1. No. of guinea pig; 2. Sensitization, antigen; 3. Desensitization;
4. Reacting injection; 5. Antigen; 6. First injection; 7. Reaction;
8. Second injection; 9. Third injection; 10. Dose of protein, mg; 11.
Liver cell nuclei of normal rats; 12. Liver cell nuclei of irradiated
rats; 13. Liver cell cytoplasm of normal rats; 14. Liver cell cyto-
plasm of irradiated rats.

tissues from six-10 animals. Nevertheless, this does not exclude the possibility of explaining the reason for the antigenic differences between the tissues of irradiated and normal animals by antigenic differences between the tissues of animals which did not belong to pure strains. As an objection to this, data may be presented which are inexplicable from the viewpoint of individual antigenic differences. For example, in various preparations from the same animals different antigenic characteristics are recorded: in the liver cell nuclei there is a simplification of the antigenic structure; in the mitochondria, the same; and in the microsomes and hyaloplasm there is a complexification; in the spleen, new antigenic properties are recorded, and in the kidney they are not. If the results of the experiments depended on individual differences between animals which were not of pure strains, the same changes should have been recorded in all tissues within the limits of the same experiment, and different experiments should have given opposite results. However, in all experiments simplification of the antigenic structure was noted in the cell nuclei. For the purpose of ruling out this possibility corresponding controls may also be used, as was done in the work of L. A. Zil'ber and others (1956). However, all this cannot absolutely eliminate this objection. Punctiliousness required the performance of experiments for comparing the antigenic properties of the tissues of the same animal taken before and after irradiation. Such experiments were performed, and they confirmed the existence of the antigenic peculiarities of tissues of irradiated animals.

One group of rabbits was irradiated with a dose of 1,000 r; the second group with a dose of 1,100 r. Before irradiation blood was taken from all the animals and serum was obtained in a sterile manner.

Table 28

Antigenic Properties of Microsomes and Mitochondria of Liver Cells
Three Days after Irradiation of Rats with a Dose of 800 r

| ① № животного серии | ② Сенсиби- лизация, литргов | ③ Десенсибилизация | | | | | ④ Разрешающее введение | | |
|---------------------------|--------------------------------------|--------------------|-------------------------|--------------|-------------------------|--------------|------------------------|------------------------|--------------|
| | | ⑤ Антиген | ⑥ Первое введение | ⑦ Реакция | ⑧ Второе введение | ⑨ Реакция | ⑩ Антиген | ⑪ Доза белка, мг | ⑫ Реакция |
| 59 | Н-мик- росомы | О-мик- росомы | 7 | — | 7 | — | Н-мик- росомы | 7,5 | + |
| 60 | | | 7 | — | 7 | — | | 1,0 | — |
| 61 | | | 3,5 | — | 5 | — | | 7,5 | — |
| 62 | | | 3 | — | 4,5 | — | | 10 | ± |
| 63*** | ⑩ | ⑪ | 7 | — | 6 | — | ⑩ | 10 | — |
| 64 | О-мик- росомы | Н-мик- росомы | 6,5 | — | 6,5 | — | О-мик- росомы | 6,5 | ++ |
| 65 | | | 2,5 | — | 6,5 | — | | 6,5 | ± |
| 66 | | | 5,5 | — | 6,5 | — | | 6,5 | ± |
| 67 | | | 3,5 | — | 5,5 | — | | 4,5 | ± |
| 68*** | | | 6,5 | — | 6,5 | — | | 7,5 | — |
| 1 | Н-мито- хондрии | О-мито- хондрии | 2 | — | 4 | — | Н-мито- хондрии | 5 | + |
| 2 | | | 2 | — | 4 | — | | 4 | ± |
| 3 | | | 4 | — | 5 | — | | 5 | ± |
| 4 | | | 3 | — | 5 | — | | 6 | ± |
| 5 | | | — | — | — | — | | 4 | — |
| 6 | О-мито- хондрии | Н-мито- хондрии | 3 | — | 5 | — | О-мито- хондрии | 5 | — |
| 7 | | | 3 | — | 4 | — | | 6 | — |
| 8 | | | 4 | — | 6 | — | | 8 | — |
| 9 | | | 2 | — | 4 | ± | | 6 | — |
| 10 | | | 2 | — | 4 | — | | 4 | — |

*Dose of protein, 20 mg; ** Dose of protein in mg; ***During sensitization no protein injected.

1-8. Same as Table 27; 9. Dose of protein, mg; 10. H-microsomes; 11. O-microsomes; 12. H-mitochondria; 13. O-mitochondria.

Table 29

Antigenic Properties of Liver Cell Hyaloplasm of White Rats Three Days after Irradiation with a Dose of 800 r

| 1 № морской свинки | 2 Сенсибилизующий антиген | 3 Десенсибилизация | | | | | | | 4 Разрешающее выделение | | |
|-----------------------|------------------------------|-----------------------|-----------------------|--------------|-----------------------|--------------|------------------------|---------------|----------------------------|----------------------|---------------|
| | | 5 Антиген | 6 Первое выделение | 7 Реакция | 8 Второе выделение | 9 Реакция | 10 Третье выделение | 11 Реакция | 12 Антиген | 13 Доза белка, мг | 14 Реакция |
| 49*** | Н-цитоплазма (11) | О-цитоплазма (12) | 3,5 | — | 6 | — | 6 | — | Н-цитоплазма (11) | 6,5 | — |
| 50 | | | 2,5 | ± | 4,5 | ± | 5,5 | — | | 7 | — |
| 51 | | | 3,5 | ++ | 4,5 | — | — | — | | 8 | — |
| 52 | | | 3,5 | ++ | 4,5 | + | 5,5 | — | | 6 | — |
| 53 | | | 3,5 | ++ | 6 | — | — | — | | 7 | — |
| 54*** | О-цитоплазма (12) | Н-цитоплазма (11) | 4 | — | 7 | — | 7 | — | О-цитоплазма (12) | 6,5 | — |
| 55 | | | 3 | + | 7 | ++ | — | — | | 6,5 | ± |
| 56 | | | 4 | + | 6 | — | — | — | | 6,5 | ++ |
| 57 | | | 3 | + | 6 | — | — | — | | 6,5 | ++ |
| 58 | | | 4 | ++ | 6 | — | — | — | | 6,5 | — |

*Dose of protein, 10 milligrams; **Dose of protein in milligrams; ***No protein injected.

1-10. Same as Table 27; 11. H-cytoplasm; 12. O-cytoplasm.

At various times after irradiation blood was again taken, and a group of guinea pigs was sensitized with the serum of each rabbit (subcutaneously in a dose of 0.5 cc). After 18-21 days the guinea pigs were used for the reaction of anaphylaxis with desensitization. During this time the sera were kept at +2-0° C. For all operations (sensitization, desensitization, reacting injection) sera of the same animal were used, taken before and after irradiation. In Table 34 the results of experiments are presented attesting to the change in the antigenic properties of the blood serum after irradiation.

Table 30

Antigenic Properties of Isolated Structural Cell Components of the Intestinal Mucosa Two Days after Irradiation of Rats with a Dose of 2,000 r

| ① № муреской спинки | ② Сенсиби- лизация, мг/мл* | ③ Антиген | Десенсибилизация** | | | | ⑧ Третье введение | Разрешающее введение | | |
|---------------------------|-------------------------------------|-------------------|-------------------------|--------------|-------------------------|--------------|-------------------------|----------------------|----------------------|--------------|
| | | | ④ Первое введение | ⑤ Реакция | ⑥ Второе введение | ⑦ Реакция | | ⑨ Антиген | ⑩ Доза бел- ка | ⑪ Реакция |
| 1 | О-ядра | Н-ядра | 1.5 | — | 3 | — | | О-ядра | 4 | — |
| 2 | | | 1.5 | — | 2 | — | | | 3 | — |
| 3 | ⑪ | ⑫ | 2 | — | 4 | — | | ⑪ | 5 | — |
| 4 | | | 1.5 | — | 4 | — | 4 | | 5 | — |
| 5 | Н-ядра | О-ядра | 1.5 | — | 3 | — | | Н-ядра | 4 | ++ |
| 6 | ⑫ | ⑪ | 2 | ++ | 4 | ++ | 4 | | 4 | ++ |
| 7 | | | 1.5 | ++ | 4 | — | | ⑫ | 3 | ++ |
| 8 | | | 2 | ++ | 3 | — | | | 4 | ++ |
| 9 | О-мито- хондри | Н-мито- хондри | 2.4 | ± | 2.5 | — | | О-мито- хондри | 2.5 | ++ |
| 10 | | | 2.4 | ++ | 2.0 | — | | | 2.0 | ++ |
| 11 | Н-мито- хондри | О-мито- хондри | 1.5 | ++ | 2.5 | — | | Н-мито- хондри | 2.5 | ++ |
| 12 | | | 1.5 | ± | 2.5 | — | | | 2.5 | ++ |
| 13 | О-мик- росомы | Н-мик- росомы | 1 | — | 2 | — | | О-мик- росомы | 3 | — |
| 14 | | | 2 | — | 3 | ± | 3 | | 3.5 | — |
| 15 | ⑮ | ⑯ | 2 | — | 2.5 | — | | ⑮ | 2.5 | — |
| 16 | Н-мик- росомы | О-мик- росомы | 2 | — | 2.5 | — | | Н-мик- росомы | 3 | — |
| 17 | | | 3 | — | 3.5 | — | | | 4 | — |
| 18 | ⑯ | ⑮ | 1 | — | 2 | — | | ⑯ | 2.5 | ± |

*Dose of protein, 10 milligrams; **Dose of protein in milli-grams; ***No reaction to third injection.

1-10. Same as for Table 27; 11. O-nuclei; 12. H-nuclei; 13. O-mito-chondria; 14. H-mitochondria; 15. O-microsomes; 16. H-microsomes

Table 31

Antigenic Properties of Some Whole Tissues Two Days after Irradiation
of Rats with a Dose of 2,000 r

| № жабры испы- тания | Сенсиби- лизация антигеном | Десенсибилизация *** | | | | | | Разрешающее введение | | |
|---------------------------|----------------------------------|--------------------------|--------------------|---------|--------------------|---------|-----------------------|--------------------------|--------|---------|
| | | Антиген | Первое введение | Реакция | Второе введение | Реакция | Третье введение ** | Антиген | Доза * | Реакция |
| 1 | (11) О-кровь | (12) Н-кровь | 0,1 | ++ | 0,1 | ± | 0,1 | (11) О-кровь | 0,15 | ++ |
| 2 | | | 0,025 | ++ | 0,1 | ± | 0,15 | | 0,15 | ++ |
| 3 | | | 0,012 | + | 0,05 | + | 0,1 | | 0,1 | ++ |
| 4 | | | 0,012 | + | 0,05 | + | 0,1 | | 0,1 | ++ |
| 5 | | | 0,015 | + | 0,05 | + | 0,1 | | 0,1 | ++ |
| 6 | (12) Н-кровь | (11) О-кровь | 0,025 | ++ | 0,1 | — | — | (12) Н-кровь | 0,1 | — |
| 7 | | | 0,012 | ++ | 0,1 | ++ | 0,1 | | 0,15 | — |
| 8 | | | 0,012 | ++ | 0,1 | — | — | | 0,1 | — |
| 9 | | | 0,012 | ++ | 0,1 | — | — | | 0,1 | — |
| 10 | | | 0,01 | ++ | 0,1 | — | — | | 0,1 | — |
| 1 | (13) О-селе- зенка | (14) Н-селе- зенка | 0,02 | + | 0,04 | — | — | (13) О-селе- зенка | 0,06 | ± |
| 2 | | | 0,02 | ++ | 0,04 | — | — | | 0,04 | ++ |
| 3 | | | 0,02 | + | 0,04 | + | 0,05 | | 0,04 | ± |
| 4 | | | 0,04 | ++ | 0,06 | — | — | | 0,08 | ++ |
| 5 | (14) Н-селе- зенка | (13) О-селе- зенка | 0,02 | + | 0,04 | — | — | (14) Н-селе- зенка | 0,06 | — |
| 6 | | | 0,01 | ++ | 0,02 | — | — | | 0,02 | — |
| 7 | | | 0,02 | ++ | 0,04 | — | — | | 0,04 | — |
| 8 | | | 0,01 | ++ | 0,03 | — | — | | 0,02 | — |
| 9 | | | 0,01 | + | 0,03 | — | — | | 0,02 | — |
| 1 | (15) О-почка | (16) Н-почка | 0,02 | + | 0,04 | — | — | (15) О-почка | 0,04 | — |
| 2 | | | 0,01 | ± | 0,03 | — | — | | 0,04 | — |
| 3 | | | 0,02 | + | 0,03 | — | — | | 0,04 | ± |
| 4 | | | 0,02 | ± | 0,04 | — | — | | 0,04 | — |
| 5 | (16) Н-почка | (15) О-почка | 0,02 | ++ | 0,04 | — | — | (16) Н-почка | 0,06 | + |
| 6 | | | 0,01 | ++ | 0,03 | — | — | | 0,02 | — |
| 7 | | | 0,01 | ++ | 0,04 | — | — | | 0,04 | ± |
| 8 | | | 0,005 | ± | 0,02 | ± | — | | 0,02 | — |

*Dose of protein for blood, 1 gr; for spleen and kidneys, 0.2 gr;

** Dose in grams; *** No reaction to the third injection.

[Legend continued next page]

irradiated organism. These data do not negate the possibility of a direct denaturing influence of radiation on proteins. They do not repudiate the participation of the first two mechanisms of change in the antigenic properties of tissues after irradiation either: penetration of exogenous antigens into the internal medium of the organism and redistribution of tissue antigens after irradiation. However, since these two mechanisms do not explain all the facts observed, we are forced to recognize the importance of distortion of protein biosynthesis and biosynthesis of associated substances after irradiation as the reason for the occurrence of antigenic peculiarities of tissues after radiation injury.

5. The Specificity of Change in the Tissue Proteins

The problem of specificity or lack of specificity of the change in the antigenic properties of tissues after the effect of ionizing radiation on the body has been solved in experiments of comparing tissue antigens of irradiated animals with antigens from animals suffering from an inflammatory process or burn sickness. For the purpose of comparing the antigenic properties of the sera of animals with radiation sickness and inflammation a comparison was made of serum from the same individual. For this purpose, blood was taken from a rabbit, and normal serum (CH) was obtained. After this, by means of the intradermal injection of 0.2 cc of the Staphylococcus aureus No 209 a focus of inflammation was created. Two days after the infection blood was taken and serum was obtained from the animal with inflammation (CB). After a week, when the inflammatory process had ended, the rabbit was irradiated on an EGO-2 apparatus with a dose of 1100 r, and after two days serum of the irradiated rabbit (CO) was obtained. Therefore, we had three samples of serum from the same animal - CH, CB and CO. There were four such rabbits in all. These samples were compared in an experiment of active anaphylaxis with desensitization on guinea pigs. The sera were obtained in a sterile manner and kept at 0° C. In Table 43 the results of four experiments are presented. They all showed the same thing: CB eliminates sensitization created by CO and vice versa. In other words, unusual antigens circulating in the blood after irradiation and in the presence of an inflammatory process are identical. Similar results were obtained in the experiment of comparing the

antigenic properties of serum of the irradiated rabbit with the serum of a burned rabbit. They were irradiated on an EGO-2 apparatus with a dose of 2000 r. The blood was taken two days after irradiation. The burn of the shaved lateral surface of the trunk, 5 x 7 centimeters in area, was made with a gas burner flame applied for 45 seconds. Blood was taken the next day.

In Table 44 the results of the experiment are presented. The serum of the burned rabbit completely eliminates the sensitization created in guinea pigs by the serum of the irradiated rabbit, attesting to the absence of distinctive antigens, different from the "burn" antigens, in the blood of the latter.

These experiments do not rule out the possibility of existence of antigens specific for radiation injury alone in the tissues rather than in the serum. For the purpose of solving this problem a comparison was made of the antigenic properties of the intestinal mucosal tissue of irradiated rats and rats with an intestinal burn. For this purpose male rats of the Wistar strain were used. An animal irradiated with a dose of 2000 r on an EGO-2 apparatus was killed after two days. The intestinal burn was made by means of intraperitoneal injection of 5 cc of physiological saline solution heated to 65° C. The animal was killed after 24 hours. The intestine was markedly thickened with focal hemorrhages. The intestinal mucosa was ground up in physiological saline solution, 1:10 and centrifuged at a rate of 1500 revolutions per minute. The supernatant was utilized as an antigen in the experiment of active anaphylaxis with desensitization.

The results of the experiment, presented in Table 45, show that in the burned intestine all antigens are present which appear in this tissue after irradiation: sensitization created by the intestine of the irradiated rat and not eliminated with desensitization by normal intestine, is completely eliminated when the preparation from the burned intestine, in which more antigenic complexes are found than in the irradiated intestine, is injected.

Therefore, at the times of radiation sickness studied (one-two days) the changes in the antigenic properties of tissues recorded are nothing specific of radiation injury but can be found in the tissues in other pathological states, for example, in burns or when an inflammatory process is present.

Therefore, the study of the antigenic characteristics of whole tissues and isolated cell structures (nuclei, mitochondria, microsomes)

Table 43

Comparison of the Antigenic Properties of Sera of Irradiated Rabbits
and Rabbits with Inflammatory Processes

| ① Сенсибилизация | ② № кролика | ③ № сыворотки | ④ Десенсибилизация | | | | | | | ⑤ Раздражающее введение | | |
|---------------------|----------------|------------------|--------------------|----------------------|--------------|----------------------|--------------|----------------------|--------------|-------------------------|-----------|--------------|
| | | | ⑤ антиген | ⑥ первое введение | ⑦ реакция | ⑧ второе введение | ⑨ реакция | ⑩ третье введение | ⑪ реакция | ⑫ антиген | ⑬ доза | ⑭ реакция |
| CO | 237 | 1 | CH | 0.05 | + | 0.07 | — | | | CB | 0.08 | + |
| | | 2 | | 0.07 | ++ | 0.08 | — | | | | 0.08 | ++ |
| | | 3 | | 0.05 | ++ | 0.07 | — | | | | 0.07 | ++ |
| | | 4 | | 1,1 | ++ | 0,1 | — | | | | 0,1 | ++ |
| | | 5 | | 0.07 | +++ | 0.08 | — | | | | 0,1 | + |
| | 279 | 6 | CH | 0.025 | ++ | 0.03 | — | | | CB | 0.03 | +++ |
| | | 7 | | 0.025 | + | 0.03 | — | | | | 0.04 | ++ |
| | | 8 | | 0.025 | +++ | 0.02 | — | | | | 0.025 | +++ |
| | | 9 | | 0.025 | ++ | 0.03 | — | | | | 0.03 | ++ |
| | | 10 | | 0.05 | +++ | 0.06 | — | | | | 0.05 | ± |
| | 210 | 11 | CH | 0.025 | +++ | 0.03 | — | | | CB | 0.03 | ++ |
| | | 12 | | 0.025 | +++ | 0.025 | — | | | | 0.025 | ++ |
| | | 13 | | 0.025 | +++ | 0.025 | — | 0.03 | — | | 0.03 | ++ |
| | | 14 | | 0.025 | +++ | 0.025 | ± | 0.03 | — | | 0.025 | + |
| CO | 237 | 15 | CB | 0.05 | +++ | 0.03 | ± | 0.07 | — | CO | 0.07 | — |
| | | 16 | | 0.5 | +++ | 0.03 | — | | | | 0.08 | — |
| | | 17 | | 0.5 | +++ | 0.01 | — | | | | 0.08 | — |
| | | 18 | | 0.025 | ++ | 0.08 | — | | | | 0.08 | — |
| | | 19 | | 0.025 | + | 0.05 | — | | | | 0,1 | — |
| | 279 | 20 | CB | 0.05 | +++ | 0.06 | — | | | CO | 0.06 | — |
| | | 21 | | 0.025 | +++ | 0.03 | — | | | | 0.03 | ± |
| | | 22 | | 0.03 | +++ | 0.05 | — | 0.05 | — | | 0.06 | + |
| | | 23 | | 0.025 | +++ | 0.03 | — | | | | 0.04 | — |
| | 210 | 24 | CB | 0.025 | + | 0.025 | ± | 0.03 | — | CO | 0.03 | — |
| | | 25 | | 0.03 | +++ | 0.03 | — | | | | 0.03 | — |
| | | 26 | | 0.03 | ++ | 0.03 | — | | | | 0.03 | — |
| | | 27 | | 0.025 | +++ | 0.025 | — | | | | 0.03 | — |
| | | 28 | | 0.025 | +++ | 0.025 | — | | | | 0.025 | — |

continued next page

| ① Сенсибилизация | ② № пробы | ③ № свинки | ④ Десенсибилизация | | | | | | ⑩ Разрежающее введение | | |
|---------------------|--------------|---------------|-----------------------|----------------------|--------------|----------------------|--------------|----------------------|---------------------------|----------------|--------------|
| | | | ⑤ антиген | ⑥ первое введение | ⑦ реакция | ⑧ второе введение | ⑨ реакция | ⑪ третье введение | ⑫ антиген | ⑬ доза, cc. | ⑭ реакция |
| CB | 237 | 29 | CO | 0,06 | +++ | 0,06 | — | | CB | 0,05 | — |
| | | 30 | | 0,025 | ± | 0,03 | — | | | 0,03 | — |
| | | 31 | | 0,025 | ± | 0,03 | — | | | 0,03 | — |
| | | 32 | | 0,05 | +++ | 0,06 | — | | | 0,07 | — |
| | | 33 | | 0,05 | ++ | 0,06 | — | | | 0,07 | — |
| CB | 270 | 34 | CO | 0,025 | +++ | 0,025 | — | | CB | 0,025 | — |
| | | 35 | | 0,025 | +++ | 0,025 | — | | | 0,025 | — |
| | | 36 | | 0,025 | +++ | 0,025 | — | | | 0,025 | — |
| | | 37 | | 0,03 | +++ | 0,03 | — | | | 0,04 | — |
| | | 38 | | 0,025 | ++ | 0,025 | — | | | 0,025 | — |
| | 270 | 39 | CH | 0,025 | +++ | 0,025 | — | | CB | 0,025 | + |
| | | 40 | | 0,025 | ++ | 0,025 | — | | | 0,025 | + |
| | | 41 | | 0,03 | +++ | 0,03 | — | | | 0,03 | + |
| | | 42 | | 0,025 | ++ | 0,025 | — | | | 0,025 | + |
| | | 43 | | 0,025 | ++ | 0,025 | — | | | 0,025 | + |

* dose in cc.

Key: + occasional scratching of the nose and sneezing; + frequently repeated scratching of the nose and sneezing; ++, the same but more marked and cough; +++, scratching of the nose, sneezing, cough, involuntary defecation and urination, convulsions; + + + +, death occurs; - no reaction. 1. sensitization; 2. number of rabbits; 3. number of guinea pigs; 4. desensitization; 5. antigen; 6. first injection; 7. reaction; 8. second injection; 9. third injection; 10. reacting injection; 11. antigen; 12. dose, cc.

of irradiated animals made it possible to determine the existence of two types of changes: the appearance of a new antigenic quality and disappearance of some of the normal antigens. The changes pertain to the organ and organoid specificity; species antigenic specificity of tissues is maintained. The new antigenic qualities are not specific for radiation injury alone. The reason for the change in the antigenic properties of the tissues is not only the direct denaturing effect of radiation but also subsequent disorders of protein metabolism. The altered antigens circulate in the blood of irradiated animals.

Table 44

Comparison of Antigenic Properties of Sera of Irradiated Rabbit and Rabbit with Skin Burn

| ① Сенси- билиза- ция | ② № свинки | ③ Десенсибилизация | | | | | ⑤ Разрешающее введение | | |
|---|---------------|--------------------|-------------------------|--------------|-------------------------|--------------|------------------------|-----------|--------------|
| | | ④ антиген | ⑤ первое введение | ⑥ реакция | ⑦ второе введение | ⑧ реакция | ④ антиген | ⑨ доза | ⑩ реакция |
| ⑩ Сыво- ротка облучен- ного кролика в дозе 1,0 мл под- кожно | 1 | Сыво- ротка | 0,025 | ++ | 0,04 | — | ⑬ Сыво- ротка | 0,04 | — |
| | 2 | ротка | 0,04 | +++ | 0,05 | — | ротка | 0,06 | — |
| | 3 | кролика | 0,025 | +++ | 0,05 | — | облучен- ного | 0,06 | — |
| | 4 | с ожогом | 0,01 | + | 0,025 | — | кролика | 0,05 | — |
| | 5 | ⑪ | 0,01 | ++ | 0,025 | — | 0,06 | — | — |
| | 6 | Сыво- ротка | 0,025 | ++ | 0,05 | — | ⑬ Сыво- ротка | 0,05 | — |
| | 7 | ротка | 0,01 | +++ | 0,04 | — | ротка | 0,06 | — |
| | 8 | кролика | 0,01 | +++ | 0,03 | — | нормаль- ного | 0,04 | — |
| | 9 | с ожогом | 0,025 | ++++ | — | — | кролика | 0,07 | — |
| | 10 | ⑪ | 0,02 | +++ | 0,03 | — | — | — | — |
| | 11 | Сыво- ротка | 0,01 | — | 0,025 | — | Сыво- ротка | 0,04 | + |
| | 12 | ротка | 0,025 | +++ | 0,04 | — | ротка | 0,06 | +++ |
| | 13 | нормаль- ного | 0,02 | + | 0,04 | — | кролика | 0,05 | + |
| | 14 | с ожогом | 0,02 | ++ | 0,05 | — | с ожогом | 0,06 | ± |
| | 15 | кролика ⑬ | 0,025 | ++ | 0,04 | — | ⑪ | 0,04 | ± |
| | 16 | Сыво- ротка | 0,025 | ++ | 0,04 | — | Сыво- ротка | 0,05 | + |
| | 17 | ротка | 0,025 | + | 0,04 | — | ротка | 0,06 | ± |
| | 18 | нормаль- ного | 0,04 | +++ | 0,04 | — | облучен- ного | 0,07 | + |
| | 19 | с ожогом | 0,01 | +++ | 0,06 | — | кролика | 0,01 | ± |
| | 20 | кролика ⑬ | 0,02 | ++ | 0,04 | — | ⑫ | 0,04 | + |

* dose in cc. 1. sensitization; 2. number of guinea pigs; 3. desensitization; 4. antigen; 5. first injection; 6. reaction; 7. second injection; 8. reacting injection; 9. dose, cc; 10. serum of irradiated rabbit in a dose of 1.0 cc subcutaneously; 11. serum of rabbit with a burn; 12. serum of irradiated rabbit; 13. serum of normal rabbit.

Table 45

Comparison of Antigenic Properties of Intestinal Mucosa of Irradiated Rat and Rat with Intestinal Burn

| ① Сенсиби- лизация | ② № серии | ③ Десенсибилизация | | | | | ④ Разрешающее введение | | |
|---|--------------|--------------------|-------------------------|--------------|-------------------------|--------------|------------------------|---------------|--------------|
| | | ④ антиген | ⑤ первое введение | ⑥ реакция | ⑦ второе введение | ⑧ реакция | ④ антиген | ⑨ доза, мл | ⑩ реакция |
| ⑩ Слизи- стая кишеч- ника облу- ченной крысы «Вистар» | 1 | ⑪ | 0,25 | ++ | 0,25 | — | ⑬ | 0,25 | — |
| | 2 | Слизи- стая | 0,3 | +++ | 0,25 | — | Слизи- стая | 0,3 | — |
| | 3 | крысы | 0,2 | +++ | 0,25 | — | облу- ченной | 0,3 | — |
| | 4 | «Вистар» | 0,3 | ++++ | — | — | крысы | — | — |
| | 5 | с ожогом | 0,2 | + | 0,25 | — | «Вистар» | 0,25 | — |
| | 6 | | 0,25 | + | 0,25 | — | | 0,25 | — |
| | 7 | | 0,25 | + | 0,25 | — | | 0,3 | ± |
| | 8 | ⑫ Нор- мальная | 0,25 | +++ | 0,25 | — | ⑬ Слизи- стая | 0,25 | ± |
| | 9 | слизи- стая | 0,25 | ++ | 0,25 | — | облу- ченной | 0,25 | ± |
| | 10 | крысы | 0,25 | ± | 0,25 | — | крысы | 0,3 | ± |
| | 11 | «Вистар» | 0,3 | + | 0,25 | — | «Вистар» | 0,2 | ± |
| | 12 | | 0,2 | ± | 0,25 | — | | 0,2 | + |
| | 13 | | | | | | | | |
| ⑪ Слизи- стая крысы «Вистар» с ожогом | 14 | ⑬ Слизи- стая | 0,3 | +++ | 0,25 | — | ⑪ Слизи- стая | 0,3 | ++ |
| | 15 | облу- ченной | 0,2 | ± | 0,25 | — | крысы | 0,35 | + |
| | 16 | крысы | 0,25 | ++ | 0,25 | — | «Вистар» | 0,35 | +++ |
| | 17 | с ожогом | 0,25 | + | 0,25 | — | с ожогом | 0,25 | ± |
| | 18 | «Вистар» | 0,25 | ± | 0,25 | — | | 0,25 | + |

* dose in cc.

1-9. same as for Table 44; 10. intestinal mucosa of irradiated Wistar rat; 11. mucosa of Wistar rat with burn; 12. normal mucosa of Wistar rat; 13. mucosa of irradiated Wistar rat.

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Chapter VI

C-REACTIVE PROTEIN AND RADIATION SICKNESS

1. The Nature of C-Reactive Protein

The discovery of C-reactive protein (CRP) -- a new antigenic substance of the animal organism -- was another achievement of noninfectious immunology which was incorporated directly into practice. Recent years have been marked by extensive incorporation of the CRP test in clinics.

An important laboratory index of the dynamics of inflammatory and destructive processes, the CRP is also of great theoretical interest, since this protein is a pathological, endogenously occurring substance with a new antigenic specificity, not characteristic of normal proteins. At the present time in the world literature hundreds of experimental studies and clinical observations have been published on CRP. A number of reviews of the literature, including ours, have appeared in recent years (Taylor, 1957; Manolin, Rabinovici, 1958; F. L. Bukh, 1958; A. L. Yampol'skiy, 1958; R. V. Petrov and Ye. N. Kabakov, 1959; A. P. Sleptsov, 1960).

The first report on this subject was made by Tillet and Francis in 1930, who described the so-called "acute phase protein" which appeared in the serum of people during the acute period of coccal infections and rheumatic fever and which gave a nonspecific precipitation with the somatic C-polysaccharide of pneumococci. After that, CRP was found in children in the acute phase of infectious diseases caused by bacteria of the colon-typhoid group (Ash, 1933). Further studies showed that CRP is not specific of any disease but rather appears in the blood in the most varied pathological processes characterized by the presence of inflammatory or destructive changes in the tissues. Thereby, its accumulation and disappearance in a number of cases more accurately depict the course of the pathological process than such reactions as the sedimentation rate or change in the body temperature. Sometimes the test for CRP in the blood of patients can prove to be a decisive factor in early diagnosis. For example, in the case of myocardial infarction this substance is always found 24-36 hours after the onset

of the disease, and a negative CRP test attests to the absence of myocardial infarction beyond a doubt (Kroop, Shackman, 1954; Roantree, Rantz, 1955).

The capacity of the "pathological protein" described for reacting with C-polysaccharide also accounted for its name, C-reactive protein. Study of the antigenic specificity of CRP showed not only its difference from normal blood antigens but also the antigenic identity of various CRP preparations obtained from different patients with different diseases (L. M. Khay, 1958; Libretty and others, 1957; Gautier, Scheidegger, 1957). In our experiments the antigenic similarity of human and *Macacus rhesus* CRP was established (R. V. Petrov, A. S. Petrova, V. V. Shikhodyrov, 1959). Lofstrom (1944), Anderson and McCarty (1951) found and isolated CRP in a crystalline form from the blood of rabbits killed 48 hours after intradermal infection with pneumococcus type I or 12-18 hours after the injection of typhoid vaccine. However, for this the authors had to prepare a pneumococcus polysaccharide (the so-called C-polysaccharide) in a special way. The C-reactive protein was obtained in a crystalline form (McCarty, 1957; Anderson, McCarty, 1951).

MacLeod and Avery (1941), McCarty (1947), Wood and McCarty (1951) showed that the detection of CRP by the precipitation test with specific antiserum is a more sensitive method than the reaction with C-polysaccharide. This method is most common at the present time. We have also used it in our studies.

CRP is found not only in the blood but also in the pleural, ascitic and spinal fluids (McCarty, 1947; Muscolino, Bellomo, 1958). Dalfabbro and Butto (1958) found CRP in the urine of patients with neuropathy. Sepvi and Novelletto (1957) showed that in the spinal fluid in some neurological diseases CRP is found more often in the blood. As has been mentioned above, CRP, absent from the blood of healthy people, appears in different diseases associated with inflammation and tissue destruction. In this group we have coccal infections and rheumatic fever (Tillett and Francis, 1930; Anderson and McCarty, 1950; Wood and McCarty, 1951), infection with the colon-typhoid group (Ash, 1933), malignant tumors (Hedlund, 1947; Roantree and Rantz, 1955), cardiovascular diseases, diseases of the digestive tract, respiratory organs, virus and bacterial infections (Hedlund, 1947 and others), skin diseases (Zina, Bonu, 1957) and others. Comparison of the dynamics of occurrence of CRP

with other nonspecific laboratory indices (sedimentation rate, temperature, leucocyte reaction, seromucoid, protein composition of the blood and others) made it possible to assert that CRP more accurately depicts the dynamics of development of the pathological process (Abernethy, 1937; Hedlund, 1947; Anderson and McCarty, 1957; Gal, Malteni, 1955; Shetlar and others, 1955; Dawson, 1957; Dimmich, 1957; Kroop, Shackman, 1954).

Schiffelin and company, which puts out diagnostic serum for determination of CRP in the blood of people, recommends the test for this protein as an infallible diagnostic test for rheumatic [rheumatoid ?] arthritis, myocardial infarction, active tuberculosis, rheumatic fever, Hodgkin's disease, Ewing's sarcoma and multiple myeloma. The absence of CRP from the blood excludes the presence of these diseases in a patient under examination. Naturally, finding it, like the other nonspecific reactions of the body, does not necessarily mean the presence of one of these diseases. However, in contrast to the other nonspecific diagnostic tests, determination of the CRP has one indisputable advantage -- it does not occur normally. Therefore, in determining the CRP we are not dealing with an increase or decrease in some factor existing normally but rather with the appearance of a new quality. At the present time, there are no sufficiently weighty data which would throw light on the pathophysiological significance of the occurrence of CRP in the blood. We do not know if this protein is some nonspecific pathogenetic link common to different diseases or an immaterial sign of the process which plays no part in the pathogenetic chain of phenomena. There are no convincing facts even with respect to the following issue: Is CRP a product of the active reaction of the body to tissue destruction or does it represent a passive result of this destruction? Some experiments by Wood (1957) indicate the fact that CRP is a product of the active reaction of reticuloendothelial cells. Our experiments with irradiated animals (R. V. Petrov and others, 1959) attest to the opposite, since they demonstrate the appearance of CRP in acute radiation sickness during the period when the reactivity of the body and its reticuloendothelial system are maximally depressed.

In the 1930's some investigators were inclined to consider CRP antibodies. However, this is absolutely incorrect. In contrast to antibodies, CRP is encountered only in the acute phase of the disease and disappears immediately after recovery. The appearance of it is

not associated with the specificity of the etiological factor and it itself possesses no antibody specificity. A necessary condition for its reaction with C-polysaccharide of pneumococci is the presence of calcium ions, which is not at all required for the antigen-antibody reaction. Finally, it has been determined that CRP can appear in cases of agammaglobulinemia, where antibody production is totally absent (Shetlar and others, 1955; Blacells-Gorina, Alvarez, 1958). In a number of studies by Wood an attempt was made to investigate the significance of occurrence of CRP in the blood and the mechanism of this process. In 1951, he investigated the effect of this protein on normal human leukocytes. It was shown that low concentrations of C-reactive protein increased the mobility of leukocytes. The same author (1953) studied the interrelationship between antibody production and C-reactive protein. Once again it was shown that C_xRP appears in response to immunization with the most diverse antigens. A correlation was established between the early occurrence of C_xRP and subsequent antibody (precipitin) production. In the same year Wood established the fact that various auxiliary agents added to vaccines and increasing antibody production also increased the production of C_xRP. Therefore, possibly, they contribute to antibody production.

In 1957, two other works appeared in which the authors continued the studies for the purpose of proving that CRP is the result of an active (and possibly compensatory) reaction of the body to tissue destruction. In the first work it was shown that the intravenous injection of small quantities of rabbit C_xRP into rabbits leads to the accumulation of tremendous quantities of it in the blood after 24 hours. If it is injected intradermally, a marked inflammatory reaction develops after 16 hours, in addition to this, which begins to disappear only after 72 hours (Wood, Montella, 1957). In the second work it was shown that blocking the reticuloendothelial system by means of intravenous injection of thorotrast inhibits or completely eliminates both reactions described above (Montella, Wood, 1957). The authors are inclined to believe that CRP is actively produced in reticuloendothelial cells. At the present time, this principle should be considered controversial. Some authors believe that CRP appears as the result of activation of proteolytic tissue enzymes (Hokoma and others, 1960; Riley and others, 1960). All the phenomena described can be explained if we assume that CRP is a passive product of tissue destruction and

inflammation and possesses antigenic qualities not characteristic of the normal. However, we have not encountered any studies in the literature which consider CRP an autoantigen. The high degree of heterology of CRP is indirectly confirmed by study of the permeability of the placental barrier to this protein: the placenta is impermeable to CRP (Philipson, Tveferas, 1957).

2. The Appearance of C-Reactive Protein in the Blood of Patients During Radiation Therapy

C-reactive protein in the blood of patients was determined by the method of precipitation in a capillary tube with immune anti-CRP-serum (I should like to take advantage of this opportunity to express my sincere appreciation to A. V. Lebedinskiy who was good enough to offer me this serum) obtained from the United States (Schiffelin and Company). The method of performing the test consisted of the following. The anti-CRP-serum was taken up in a glass capillary tube 0.4-0.8 millimeter in diameter to one-third of its height. Then patient's serum was taken up in the capillary tube for another third of its height. The contents were mixed by brief shaking and rotation. The capillary tube was set in modeling clay, placed for two hours at 37° C, and then left for the night at room temperature. In the case of a positive test a precipitate was found on the lower meniscus which had come down during the night. To some degree the reaction was evaluated quantitatively by the height of the precipitate: height of the column less than one millimeter, +; one millimeter, ++; two millimeters, +++; three millimeters, ++++; four millimeters or more, +++++. This method has been recommended by the company which puts out the diagnostic anti-CRP-serum and is considered semiquantitative. Incidentally, it should be kept in mind that the very fact that CRP appears in the blood is of great importance, and no quantitative evaluation is needed for this, because normally this protein is absent from the blood. The examination of the patients was made in the department of radiation therapy directed by M. P. Domshlak with the active participation of the department physician N. L. Melik-Pashayeva. In all, 56 patients with different tumors were investigated. In Table 46 data are presented on the diagnoses and results of the study for CRP before beginning radiation therapy.

Table 46

The Number of Positive and Negative Tests for CRP in Different Tumor Cases

| ① Результаты реакции на СРП | ④ Рак груди и железы | ⑤ Рак гортани | ⑥ Рак мочевого пузыря | ⑦ Рак пище- вода | ⑧ Рак матки | ⑨ Лимфосар- кома | ⑩ Лимфогра- нулематоз | ⑪ Гемангиома | ⑫ Базилома | ⑬ Опухоль около ушной железы | ⑭ Прочие за- болевания |
|-----------------------------------|-------------------------------|------------------|-----------------------------|------------------------|----------------|------------------------|-----------------------------|-----------------|---------------|---------------------------------------|------------------------------|
| Положительная ② | 11 | 3 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Отрицательная ③ | 28 | 2 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 3 |

1. results of tests for CRP; 2. positive; 3. negative; 4. cancer of the breast; 5. cancer of the larynx; 6. cancer of the bladder; 7. cancer of the esophagus; 8. cancer of the uterus; 9. lymphosarcoma; 10. Hodgkin's disease; 11. hemangioma; 12. basal cell carcinoma; 13. tumor of the parotid gland; 14. other diseases.

From the Table it is seen that in 40 out of 56 cases the test for CRP was negative and in 16 it was positive. Of 56 patients 41 persons were examined a second time after beginning the course of radiation therapy and of these 27 had negative reactions before irradiation and 14, positive.

Irradiation of the patients was conducted locally with X-rays with a dose rate of 15-60 r per minute. The single dose in different patients and with different sizes of the field ranged from 150 to 300 r per field. In the majority of cases the patients were irradiated every other day until a total dose of 6000-8000 r was reached, unless a radiation reaction occurred sooner and if the patient's condition allowed continuing treatment. Examination of the patients (determination of the white blood count, sedimentation rate and the test for CRP) was conducted every time on the day of irradiation. During the course of X-ray therapy the test for CRP in the blood changed in the majority of patients (Table 47).

Of the greatest interest are the 27 patients in whom there was no CRP in the blood before the beginning of radiation therapy. In 19 cases this protein appeared, and in eight cases it did not appear.

Table 47

Characterization of Patients by the Results of the Tests for CRP Before Beginning X-ray Therapy and by the Change in It During the Course of Irradiation

| ① Результаты обследования до облучения | ④ Число случаев | ⑤ Обследованы | | ⑧ Изменение реакции в процессе рентгенотерапии | | |
|---|-----------------------|----------------------|-----------------------|--|------------------------------------|--|
| | | ⑥ одно- кратно | ⑦ много- кратно | ⑨ отрица- тельная перешла в положи- тельную | ⑩ реакция не изме- нялась | ⑪ положи- тельная перешла в отрица- тельную |
| ② Отрицательные | 40 | 13 | 27 | 19 | 8 | — |
| ③ Положительные | 16 | 2 | 14 | — | 8 | 6 |

1. results of examination before irradiation; 2. negative; 3. positive; 4. number of cases; 5. examined; 6. once; 7. many times; 8. change in the reaction during X-ray therapy; 9. negative, changed to positive; 10. reaction did not change; 11. positive changed to negative.

In Tables 48 and 49 some information is given about these two groups of patients. The first group of patients was distinguished by the fact that in the majority of cases the development of a "radiation reaction" was observed in the form of acceleration of the sedimentation rate (more than 12 millimeters an hour), general weakness, nausea, skin lesions in the area of irradiation (erythema, erosions). In some patients leukopenia developed (less than 3000 white blood cells per cubic millimeter of blood). Increased in the sedimentation rate was recorded in 15 out of 19 cases; a skin reaction in 16 cases, the development of general weakness in 16 cases. In a number of cases a temperature elevation and other pathological signs were recorded.

C-reactive protein appeared in the blood at approximately the same times at which the "radiation reaction" developed -- two-four weeks after the beginning of irradiation. By this time a total dose of several thousands of roentgens had been accumulated.

Table 48

Diagnosis and Radiation Effect in Patients with a CRP Test Which Changed From Negative to Positive

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | | | | 9 |
|----|-----------------------------------|-----|----|-------|----|-------|-----------------------------------|-------------------|--|--|----|
| | | | | | | | а) Длительность периода облучения | б) Доза облучения | в) Доза облучения на 1 см ² | г) Доза облучения на 1 см ³ | |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| 14 | Рак грудной железы | 350 | 30 | 8700 | 19 | 5600 | — | — | — | — | — |
| 15 | То же | 200 | 22 | 7000 | 22 | 7000 | + | + | + | + | + |
| 16 | » после операции | 200 | 15 | 4200 | 22 | 4200 | — | — | — | — | — |
| 17 | Лимфогранулематоз | 300 | 23 | 10900 | 30 | 10900 | — | — | — | — | — |
| 18 | Рак матки после операции | 200 | 28 | 8800 | 27 | 8600 | — | — | — | — | — |
| 19 | То же | 200 | 33 | 9000 | 27 | 7000 | + | + | + | + | + |
| 20 | Рак грудной железы после операции | 200 | 27 | 6600 | 41 | 6600 | — | — | — | — | — |
| 21 | То же | 200 | 12 | 2400 | 9 | 1600 | + | + | + | + | + |
| 22 | Рак грудной железы | 200 | 12 | 2400 | 9 | 1600 | + | + | + | + | + |

1. Diagnosis; 2. area of irradiation; 3. single dose per field; 4. duration of irradiation period; 5. total dose; 6. time of appearance of CRP; 7. CRP dose after which CRP

Continuation of Table 48

| Диагноз | Зона облучения | Разовая доза в ОД, р | Длительность курса, сут | Суммарная до- за, р | Средняя до- за, р | Средняя до- за, р | Некоторые клинические показатели | | | | | Примечание |
|--------------------------------------|-------------------|-------------------------|----------------------------|------------------------|----------------------|----------------------|-------------------------------------|---------------------|-------------------|----------------------------|-------------------|--|
| | | | | | | | Анемия | Увеличе- ние РОЭ | Повыше- ние АД | Повыше- ние температуры | Общая слабость | |
| Рак грудной железы | Грудная клетка | 200 | 21 | 2000 | 21 | 2000 | - | + | - | + | + | Полос. темпера- тура 37,6° С |
| " | То же | 200 | 25 | 7000 | 17 | 2800 | + | + | + | + | + | |
| " | " | 200 | 23 | 7200 | 12 | 3800 | - | - | + | + | + | |
| Рак мочевого пузыря | Живот | 150 | 22 | 6450 | 22 | 6450 | - | + | + | + | + | Температура |
| Гемангиома позвоночника | Позво- ночник | 150 | 10 | 600 | 11 | 800 | - | + | - | + | + | Облучалась 6 ме- сяцев назад до- зой 2700 р. |
| Рак гортани | Шея | 200 | 3 | 1200 | 6 | 1200 | - | + | + | + | + | Облучалась 4 ме- сяца назад до- зой 4000 р. |
| Рак грудной железы | Грудная клетка | 200 | 22 | 7200 | 9 | 3000 | - | + | + | + | + | Температура |
| Математика | То же | 150 | 21 | 5100 | 10 | 2700 | - | - | + | + | + | Температура |
| Рак грудной железы после операции | То же | 200 | 23 | 7400 | 46 | 7400 | - | + | + | + | + | Температура |
| Лимфогранулема | Шея | 150 | 18 | 8300 | 9 | 7000 | - | + | + | + | + | Температура |
| Опухоль окологлоточной железы | " | 200 | 9 | 1400 | 24 | 1400 | - | - | - | - | - | Температура |

appeared, r; 8. some clinical indices; 9. leukopenia; 10. increased sedimentation rate; 11. skin reaction; 12. general weakness; 13. note; 14. breast cancer; 15. chest; 16. the same; 17. after operation; 18. Hodgkin's disease; 19. abdomen; 20. cancer of the uterus after operation; 21. breast cancer after operation; 22. temperature rose twice; 37.6° and 37.9° C; continuation on next page

[continued from previous page]

23. temperature rose to 37.4°C ; 24. had been irradiated one month before with a dose of 8200 r; 25. cancer of the bladder; 26. hemangioma of the spine; 27. spinal column; 28. cancer of the larynx; 29. neck; 30. matamelanoma [this is the way it reads in the text; apparently metamelanoma was intended and this possibly was an error for metastatic melanoma]; 31. lymphosarcoma; 32. parotid tumor; 33. diarrhea, temperature of 37.6°C ; 34. hematuria; 35. was irradiated six months before with a dose of 2700 r; 36. was irradiated four months before with a dose of 4000 r; 37. temperature of 38°C and herpes; 38. influenza, temperature 39°C .

In comparing this group of patients with those in whom CRP did not appear in the blood during the course of radiation therapy two facts attract attention. First of all, in the majority of cases there was no pronounced "radiation reaction" in the second group (compare the right-hand portions of Tables 48 and 49). No increase in the sedimentation rate, for example, was observed in a single patient. Secondly, more than half of the patients in this group received relatively low total doses of radiation. Comparison of these two groups of patients gives us some basis for the conclusion that the appearance of CRP in the blood is associated with the radiation effect; particularly, since among the persons investigated seven had been given X-ray therapy after surgical removal of a tumor. Nevertheless, the radiation effect, leading to the development of the "radiation reaction," was associated with the appearance of CRP. In those cases the appearance of CRP in the blood cannot be explained by the development of the neoplastic process in the body. However, this, to be sure, does not completely answer the possible objections, since in all cases the combination of at least two pathological processes is observed, of which one -- the neoplastic one -- in itself can cause the appearance of CRP; particularly since we have a group of patients made up of six persons in whom in whom the CRP recorded before the beginning of irradiation disappeared from the blood during the course of X-ray therapy.

We shall not go into the case histories of all the patients in more detail, since on the basis of these studies it is impossible to draw a final conclusion concerning the appearance of CRP in the blood as the result of the effect of ionizing radiation on the body. It can be noted only that in the majority of cases C-reactive protein does appear

Table 49

The Diagnosis and Radiation Effect in Patients With a Constantly Negative Test For CRP

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|--|---------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| Group of patients (number of patients) | Some patients | Number of patients | Number of patients | Number of patients | Number of patients | Number of patients | Number of patients | Number of patients | Number of patients | Number of patients | Number of patients | Number of patients | Number of patients |
| Group 1 (10 patients) | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Group 2 (10 patients) | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Group 3 (10 patients) | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Group 4 (10 patients) | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

1-5. same as Table 48; 6. some clinical indices; 7. leukopenia; 8. increased sedimentation rate; 9. skin reaction; 10. general weakness; 11. breast cancer (after operation); 12. breast cancer; 13. the same; 14. chest cage.

in the blood during the course of radiation therapy in tumors. In these cases the most marked reaction of the body is recorded to irradiation in the form of an increased sedimentation rate, skin lesions, and some general symptoms (weakness, nausea, and others). The CRP correlates most closely with the sedimentation rate. Whether it is the result of the effect of ionizing radiation or, particularly, whether CRP appears in the blood during radiation sickness can be answered only after experimental study of this problem in experiments on animals. We have performed such experiments on monkeys.

3. C-Reactive Protein in the Blood of Monkeys with Acute Radiation Sickness

Experiments on the study of CRP in acute radiation sickness were performed on *Macacus rhesus* monkeys. The detection of this protein was accomplished by means of anti-CRP-serum after its power of reacting with monkey CRP was established. The test, performed by the usual method in healthy monkeys also (18 animals were examined) was always negative. A positive test was found in monkeys suffering from disease: in two monkeys sick with poliomyelitis in the stage of paralysis; in one with inflammation of the lungs and in a monkey with two carious teeth and large abrasions around the eyes.

The first experiment was performed in Moscow under conditions of laboratory maintenance of the animals in the month of May (R. V. Petrov, A. S. Petrova, V. V. Shikhodyrev, 1959) on three male monkeys four years old. Two of them ("Ataman" and "Starik") were perfectly healthy and had a negative CRP test. One ("Slaby") had a faintly positive test (+), apparently conditioned by the presence of carious teeth and abrasions. The animals were irradiated with gamma-rays on an EGO-2 apparatus with a dose rate of 430 r per minute. The results of the experiments are shown in the form of three figures.

Fig. 14 represents the experiment protocol with the monkey "Starik." The development of typical postradiation leukopenia, loss of weight, elevation of body temperature on the second-fourth and seventh days after irradiation are seen. The test for CRP, performed 24 hours after irradiation, showed the maximum positive result (4+). On the second day, the reaction was also markedly positive. On the third day, CRP had disappeared from the blood and did not appear in the next five days. On the eighth day a faintly positive test was recorded (+); on the ninth, a markedly positive test (+ + + +); on the tenth day, the monkey died.

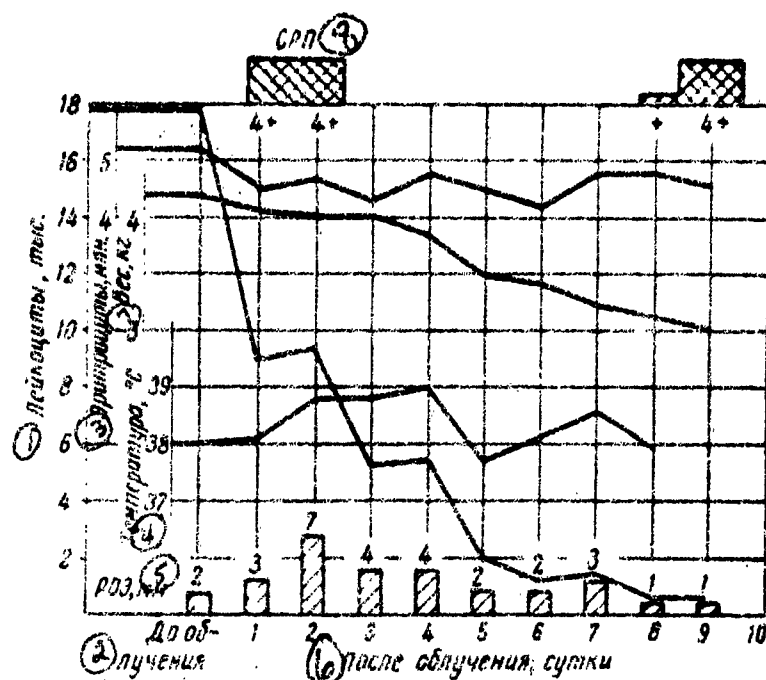


Fig. 14. Chart of the Monkey "Starik." Died on the 10th day after irradiation with a dose of 600 r. 1. white blood count, thousands; 2. before irradiation; 3. red blood count, millions; 4. temperature, degrees C; 5. sedimentation rate, mm; 6. after irradiation, days; 7. weight, kilograms; 8. CRP.

In the monkey "Slaby" (Fig. 15), just as in the previous case, the test was positive after one and two days; on the third day after irradiation CRP disappeared from the blood; on the fourth day, this protein appeared in small quantities; on the fifth, its content in the blood increased, and on the sixth it reached the level of the first day. On the seventh day the monkey died. Autopsy and histologic study of the organs of both monkeys, performed by V. V. Shikhodyrov, did not reveal the presence of any pathological process with the exception of acute radiation sickness. Culture of the intestinal contents for dysentery gave a negative result.

Radiation sickness in the monkey "Ataman" (Fig. 16), in contrast to the other two, developed particularly precipitously, and

the animal died four days after irradiation. The CRP in the blood of this monkey appeared 24 hours after irradiation and did not decrease until death. On autopsy and histologic study no additional pathological processes were found either. The cultures for dysentery were negative.

Therefore, in the blood of monkeys after irradiation of them with a certain lethal dose of gamma-rays CRP appears. In all cases it is found in maximum quantities during the two days after the radiation effect. Subsequently, C-reactive protein can disappear from the blood or remain until death occurs, which is apparently associated with greater or lesser severity of the course of radiation sickness. Before death the CRP content in the body increases again.

A second experiment (R. V. Petrov, E. K. Dzhikidze, A. S. Petrova, 1960) was performed under conditions in which the monkeys were kept in an open-air cage at the Institute of Experimental Pathology in the laboratories of L. F. Semenov and E. K. Dzhikidze. Six monkeys were studied which had been irradiated with gamma-rays (dose rate, 70 r per minute) in a dose of 614 r; two monkeys were irradiated with a dose of 480 r; and three with a dose of 315 r.

In these experiments the rapidity with which CRP appeared in the blood was studied. For this purpose, blood was investigated in the animals which had been taken one, two, three, six, nine, 12 and 24 hours after irradiation, and then every day. It was determined that in all monkeys irradiated with a dose of 614 r the CRP appears after three hours (reaction of + or ++) and reaches a maximum after six-nine hours. Of the two monkeys irradiated with a dose of 480 r, CRP was found in one case after three hours. Of the three animals irradiated with a dose of 315 r, CRP was found in two cases after three hours; in one monkey it appeared for the first time after six hours.

On Figs. 17-25 the records of all the experiments are presented, the results of which are in full agreement with those described above and permit us to draw the following conclusions:

1. When monkeys are irradiated with gamma-rays in doses of 315-614 r the CRP appears in the blood after three hours and reaches a maximum after 9-12 hours.
2. In cases of the rapid course of the radiation injury, where the monkeys live a total of four-six days, the CRP does not disappear from the blood until the animal dies.
3. In cases of a more prolonged, typical course of radiation sickness CRP disappears from the blood two-four days after irradiation.
4. Two-seven days after the first wave of occurrence of CRP

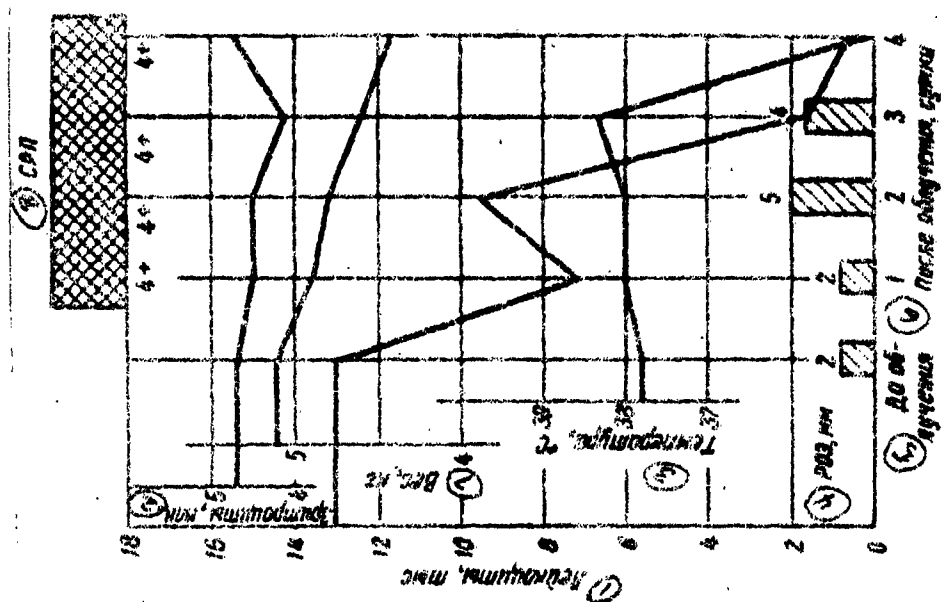


Fig. 15. Chart of the Monkey "Slavyi." Died on the seventh day after irradiation with a dose of 600 r. 1. white blood count, thousands; 2. red blood count, millions; 3. temperature, degrees C; 4. sedimentation rate, mm; 5. before irradiation; 6. after irradiation, days; 7. weight, kg; 8. CRP.

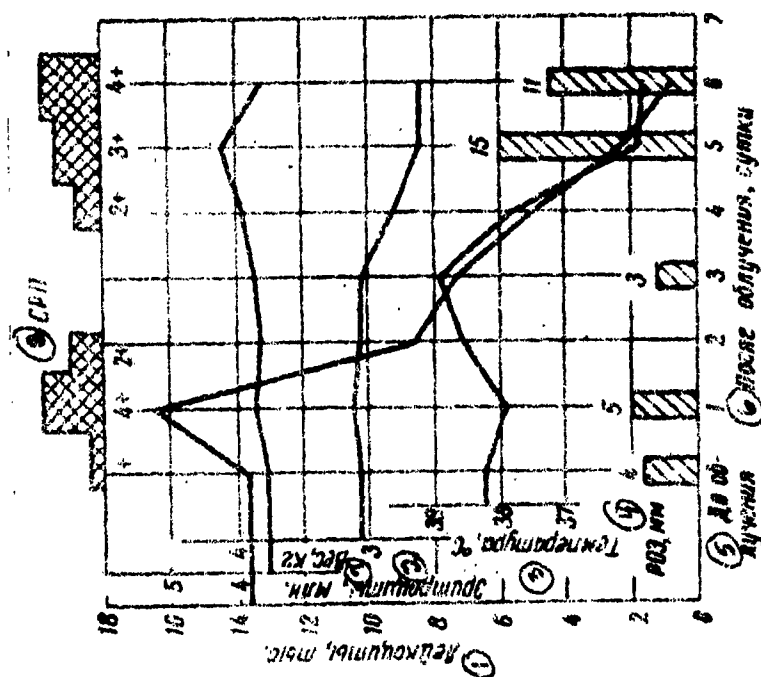


Fig. 16. Chart of the Monkey "Ataman." Died on fourth day after irradiation with dose of 600 r. 1-8. same as Fig. 15.

has terminated it always appears in the blood a second time, constituting a record of the most severe period of radiation sickness.

The majority of animals irradiated with a dose of 614 r died with signs of striking radiation sickness; the second appearance of CRP in their blood always began three-five days before death.

The latter conclusion may be of very important practical significance, because the second appearance of CRP in the blood gives a two-three-day warning of the approach of the most severe (critical) period of radiation sickness. In various organisms this period occurs at different times. Conversely, a long period of absence of CRP between the first and second waves is a favorable sign.

For the clinician the comparison of the dynamics of CRP with other laboratory indices, particularly with the sedimentation rate, may be interesting, since in other types of pathology this reaction most closely correlates with the CRP. An analysis of the charts presented in the Figures permits us to make such a comparison. A correlation with the sedimentation rate is actually observed in the majority of cases during the second phase of appearance of CRP. During the first three days after irradiation the sedimentation rate is always within normal limits. The sensitivity of the sedimentation rate to tissue destruction is apparently incomparably lower than that of the CRP test, as the result of which the process of primary tissue destruction is not recorded by means of the sedimentation rate. Aside from the possible practical interest of the conclusions presented above, the utilization of these facts for the analysis of processes of tissue destruction after irradiation is of great importance. CRP is particularly valuable for these purposes for two reasons: first of all, its dynamics depict the dynamics of the destructive processes most precisely of all the laboratory methods, and, secondly, it is a protein with antigenic qualities not characteristic of the normal. The latter is of exceptional interest to us in connection with the study of tissue antigens of the irradiated organism. Therefore, the CRP dynamics represent the dynamics of tissue destruction and appearance of substances of autoantigenic nature after irradiation.

In the previous chapter a number of data was presented attesting to the early development of destructive processes in the tissues and early change in their antigenic properties after irradiation. Studies of C-reactive protein have not only confirmed them but have also shown the early appearance of abnormal proteins in the blood after three hours. In addition, they made it possible to establish two phases of tissue destruction and of discharge of CRP into the blood: the first, directly after

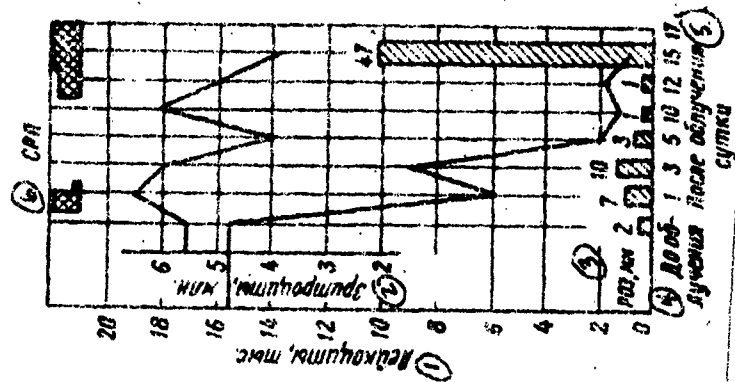


Fig. 17. Chart of the Monkey "Radzhan." Died on the 17th day after irradiation with 600 r. 1. white blood count, thousands; 2. red blood count, millions; 3. sedimentation rate, mm; 4. before irradiation; 5. after irradiation, days; 6. CRP.

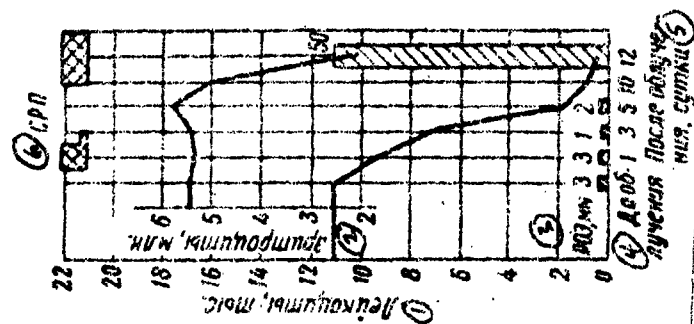


Fig. 18. Chart of the Monkey "Plum." Died on the 13th day after irradiation with a dose of 600 r. 1-6. same as Fig. 17.

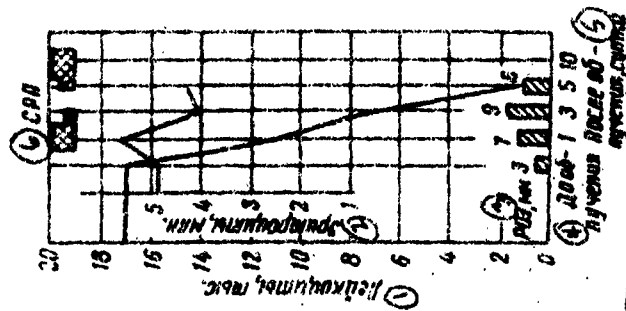


Fig. 19. Chart of the Monkey "Byuval." Died on the 13th day after irradiation with a dose of 600 r. 1-6. same as Fig. 17.

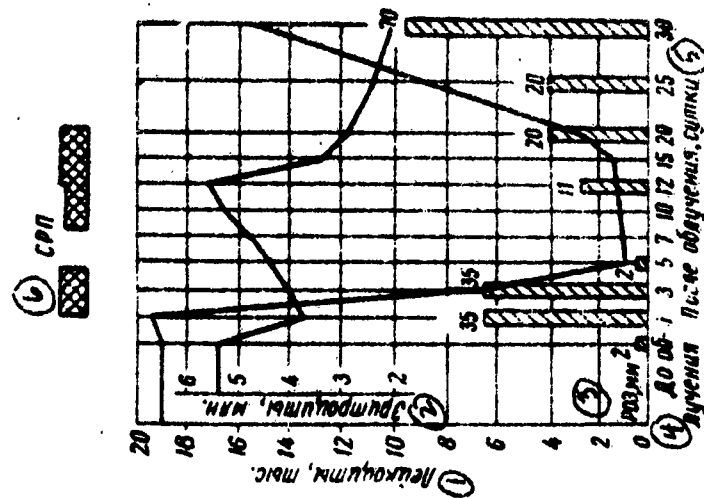


Fig. 20. Chart of the Monkey "Khorta," Irradiated with a Dose of 600 r. Survived. 1.-6. same as Fig. 17.

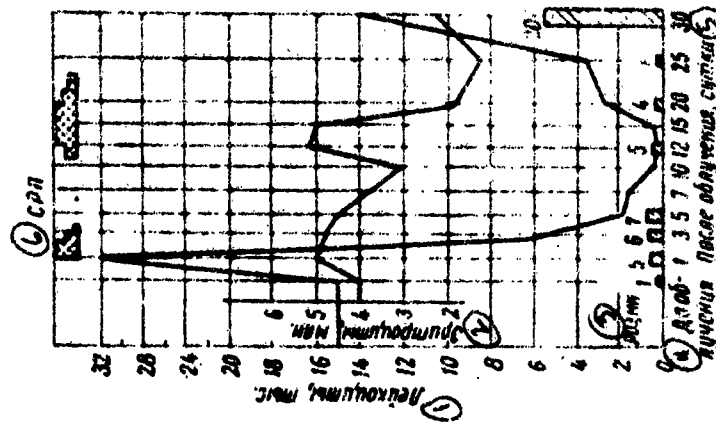


Fig. 21. Chart of the Monkey "Khariza," Irradiated with a Dose of 600 r. Survived.

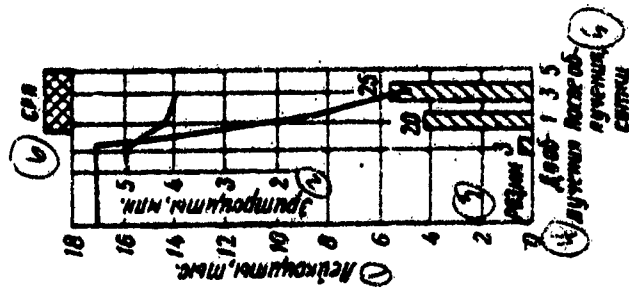


Fig. 22. Chart of the Monkey "Deringa," Died on the sixth day after irradiation with a dose of 600 r.

Fig. 23. Chart of Monkey No 3260 Irradiated with a Dose of 480 r. Survived. 1. white blood count, thousands; 2. sedimentation rate, mm; 3. CRP; 4. duration of experiment, days.

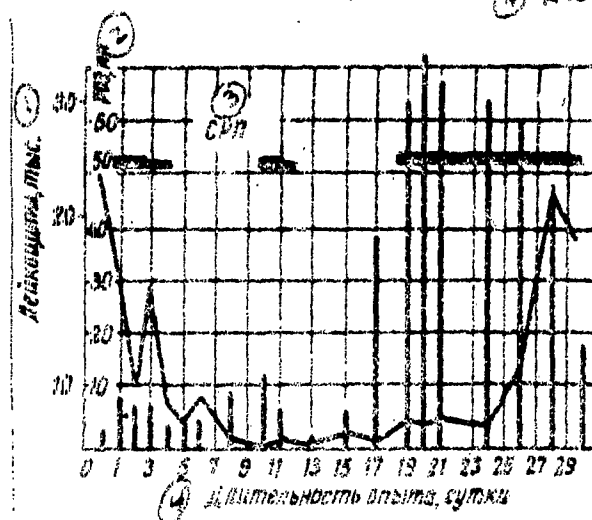
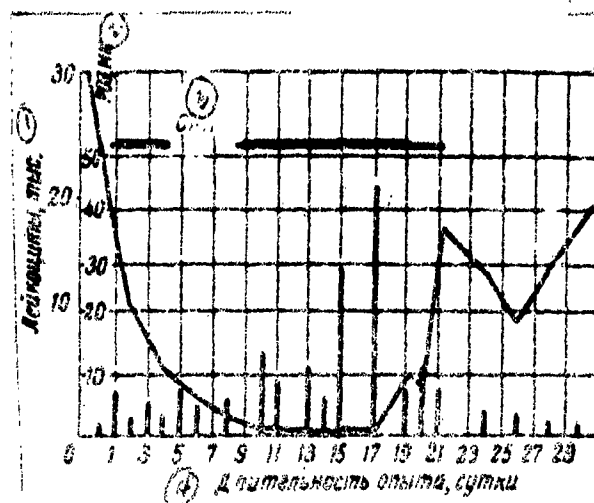
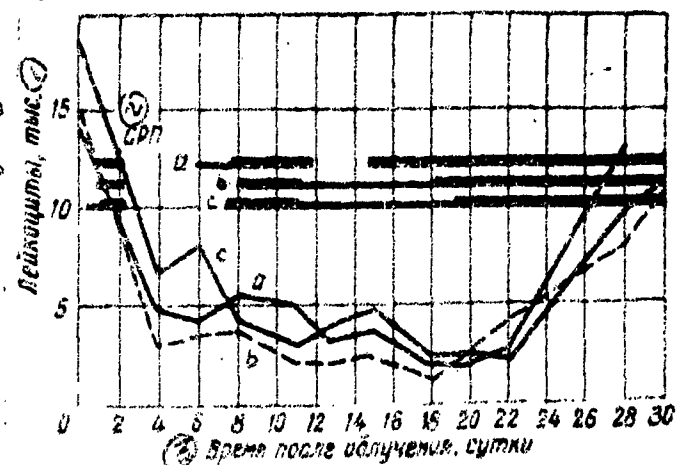


Fig. 24. Chart of Monkey No 3274 Irradiated with a Dose of 480 r. Survived. 1-4. same as Fig. 23.

Fig. 25. Chart of Monkeys Surviving After Irradiation with Dose of 315 r: a. CRP and white blood count of Monkey No 3526; b. CRP and white blood count of Monkey No 3496; c. CRP and white blood count of Monkey No 3457; 1. WBC, thousands; 2. CRP; 3. time after irradiation, days.



irradiation and the second, several days before death, or during the most severe period of radiation sickness. The first phase of destruction, which lasts two-three days, is undoubtedly associated with the direct influence of radiation on the tissues. This is evidenced by the rapidity of appearance of CRP in the blood -- after three hours -- and the brevity of this first phase, it terminates after two-three days. In the case of a precipitously occurring radiation injury, where the animal dies on the fifth-sixth day, the organism does not go further than this phase of initial tissue destruction. As far as the second phase of appearance of CRP in the blood of irradiated animals is concerned, it may be explained by mechanisms mediated through the organism and autoinfection (Wood and others, 1960).

Since CRP is an index of tissue destruction, the test for this protein can prove to be very useful in radiation therapy clinics for evaluating the injurious effect of radiation. The specific dynamics of its appearance in the blood in acute radiation sickness also make it possible to follow the rates and intensity of development of the pathological process and to orient the physician with respect to the critical period for the patient's life.

In the blood of monkeys C-reactive protein appears three hours after the effect of radiation and reaches a maximum after 9-12 hours. In cases of the rapid course of radiation sickness, where the monkeys live a total of four-six days, CRP does not disappear from the blood until death occurs. In the other cases, CRP disappears two-three days after irradiation. CRP always appears a second time two-three days before death, and in cases of recovery the second wave of appearance of CRP documents the most severe period of radiation sickness. CRP in the blood of sick people appears during X-ray therapy together with the development of the so-called radiation reaction.

Until very recently in the literature there was no information about the appearance of CRP in radiation sickness, and we were unable to compare the results of our own experiments with others. Only most recently have several reports appeared (P. A. Buzini and P. N. Kiselev, 1960; V. P. Moiseyeva, 1960; Wood and others, 1960; Riley and others, 1960). In them the rules and regulations of appearance of CRP in the blood of oncological patients during X-ray therapy and in the blood of animals irradiated with lethal and sublethal doses of radiation are shown. In acute radiation sickness, as in our observations, two phases of appearance of CRP are shown (V. M. Moiseyeva, Wood and coauthors, and Riley and coauthors).

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Chapter VII

REACTIONS OF THE BODY TO TISSUE ANTIGENS

1. Toxicity of Tissues of Irradiated Animals

The role of toxemia in the pathogenesis of radiation sickness has been repeatedly discussed in the literature (P. D. Gorizontov, 1958, 1959; N. N. Kuznetsova, 1957; E. Ya. Grayevskiy and I. M. Shapiro, 1959; V. N. Benevolenskiy, Barnes, Furth, 1943, and others). Numerous experiments with blood transfusion from irradiated animals to nonirradiated animals, cross circulation and parabiosis, a search for toxins in vitro have proved the appearance of toxic substances in the blood after irradiation of animals. Our problem is not the presentation of all the material on this subject; this material has been adequately completely discussed in the generalizing works mentioned above. It is important to emphasize the fact that the site of formation of the toxic products and their "main reservoir" (Campo and others, 1958) consist of the affected tissues rather than the blood. This is illustrated particularly clearly in the following works.

G. P. Gruzdev, N. K. Yevseyeva and V. D. Rogozkin (1958) and then Yu. D. Balika (1959), perfusing parts of the body of an irradiated animal isolated in a vascular respect with the blood of a healthy animal, showed that blood flowing out of various organs or parts of the bodies of irradiated dogs possesses different toxic effects. In these experiments the tissues of the irradiated animal were "washed out" with the blood of the healthy animal. It was determined that as early as 24 hours after irradiation the washing out of toxic substances from the tissues occurs. The maximum toxicity is recorded after three days (Yu. D. Balika). A. V. Lebedinskiy, A. S. Petrova and L. A. Buldakov (1957) established the fact that if during the first two days after irradiation all the lymph coming out of the affected parts of the bodies of dogs is drained through an exteriorized thoracic duct the signs of radiation sickness are much less pronounced.

Hemolysins found by A. S. Mochalina (1957) and studied in detail by V. N. Benevolenskiy (1957, 1958) in radiation sickness accumulate specifically in the tissues. Cytolysins against the body's own cells were found by N. N. Klemparskaya (1957) not only in the blood but also in the tissues. In the reactions of cytotoxicity

liver and leukocyte cells were used. It was shown that the maximum accumulation of leukolysins occurs at the time when the white blood counts of irradiated animals are markedly reduced. A number of data have been published showing the development of typical radiation sickness after irradiation of various parts of the body, for example, exteriorized intestinal loops (Osborn, 1956), the facial part of the head (L. F. Semenov and B. A. Fedorov, 1959), a skin flap (N. N. Kuznetsova, 1957) and others. Thereby, Osborn showed that resection immediately after irradiation of the affected intestinal loops prevents the development of radiation sickness.

The data presented above as well as ideas about the possible role of tissue destruction products entering the blood by virtue of increased permeability of histohematic barriers in the pathogenesis of radiation sickness impelled N. N. Klemparskaya in 1956 to study the toxic properties of the tissues of irradiated animals. She showed that parenteral administration of tissue homogenates to animals of the same species brings about a considerable increase in their sensitivity to the effect of ionizing radiation. After the administration of large doses to intact animals (for example, 3-10 cc of a 25 percent tissue suspension per guinea pig) a pathological process develops which in a number of cases causes death after several days. In our combined experiments (N. N. Klemparskaya, R. V. Petrov, L. I. Il'ina, 1958), performed at the suggestion of N. N. Klemparskaya, conditions of preparation and dosaging of tissue preparations were standardized.

Taking into consideration the fact that destructive processes, changes in metabolism and in antigenic properties are expressed differently not only in different organs but also in various structural elements of cells of irradiated animals, we made a study of the biological effect not only of whole tissue homogenates but also isolated microstructures (nuclei, mitochondria, microsomes and hyaloplasm) of liver and small intestinal mucosal cells of irradiated and control rabbits after parenteral administration of them to healthy animals of the same species. The tissue homogenates were prepared by means of grinding them with ten times the quantity of physiological saline solution in a high-speed blender with subsequent precipitation of intact cells and other coarsely dispersed particles by centrifugation at 1200-1500 revolutions per minute. The cell microstructures were isolated by differential centrifugation according to the method described above. The preparations were measured out according to their protein content, calculated by the Kjeldahl method. In testing the biological effects of tissues of irradiated rabbits, the animals

were sacrificed 6-24 hours after X-ray irradiation (180 kv, 20 ma, 29-30 r per minute, filters of 0.5 mm Cu plus 1.0 mm Al) or with gamma-rays of Co^{60} at a dose rate of 340 r per minute.

The biological effects observed may be divided into two types: 1) direct -- after injection -- and untypical with respect to the clinical picture, characteristic of the preparations made from small intestinal mucosa; 2) remote, observed after administration of different tissues, and different in its manifestations. The direct effect was observed only after intravenous injection of certain quantities of the preparations of intestinal mucosa. This effect was expressed in the development of shock: dyspnea, ataxic gait, urination, convulsions and death after one-three minutes. The administration of the same or much larger quantities intraperitoneally, intramuscularly or subcutaneously caused no direct effect.

In determining the minimum lethal doses of various preparations it was found that they do not possess the same biological effectiveness. It was determined that amorphous cytoplasm, that is, fluid which remains after the isolation of nuclei, mitochondria and microsomes from the cell homogenate, does not possess a shock-producing effect even when administered in a volume of 30 cc. The other intestinal mucosal cell components and the whole tissue homogenate cause rapid death of rabbits after intravenous injection of certain quantities. The minimum lethal dose (MLD) of the preparations from irradiated rabbits is several times less than that from normal rabbits. Thus, the MLD of a mucosal homogenate of an irradiated rabbit is equal to 7.5 cc; of a control, 20.0 cc. For the nuclear fraction the minimum lethal doses are equal to 2.0 and 10.0 cc respectively.

The test of the biological effect of mitochondria under similar experimental conditions showed that the minimum lethal dose of preparations from irradiated rabbits (0.5-1.0 cc) is several times less than the control (3.0 cc). Therefore, the mitochondria of intestinal cells of irradiated and normal rabbits were two-three times more toxic than the nuclei and more than seven times more toxic than the whole tissue homogenate. The biological activity of the intestinal mucosal microsomes was even greater: the minimum dose which killed the rabbit with the same shock-like symptoms was equal to 0.25-0.35 cc for preparations obtained from irradiated animals and 1.0-2.0 cc for control microsomes.

On Fig. 26 data are presented on the comparison of the minimum lethal doses of different cell organoids of intestinal mucosae of normal and irradiated rabbits. A higher degree of toxicity of intestinal mucosal tissue of irradiated animals by comparison with intact animals is found also in experiments in which

heterologous tissue is administered. We in cooperation with L. I. Il'ina showed this by determining the MLD of mitochondria from the intestinal mucosae of normal and irradiated rats on mice (L. I. Il'ina, R. V. Petrov, 1960).

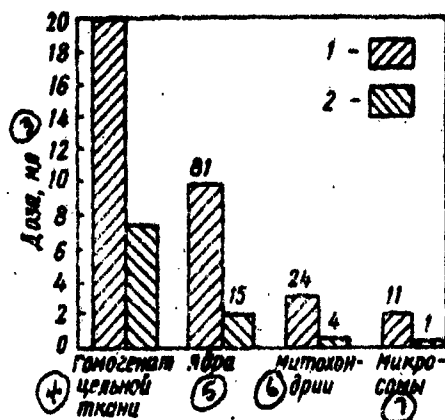


Fig. 26. Minimum Lethal Doses (Shock-Producing) of Various Preparations from the Intestinal Mucosae of Normal and Irradiated Rabbits Injected Intravenously into Healthy Rabbits. (The figures over the columns designate the doses of the preparations in milligrams of protein): 1. preparation from normal rabbits; 2. preparations from irradiated rabbits; 3. dose, cc; 4. homogenate of whole tissue; 5. nuclei; 6. mitochondria; 7. microsomes.

With the aim of finding out the mechanism of influence of tissue preparations from the intestinal mucosae of irradiated and healthy rabbits on the vital functions of intact animals, a study was made of the condition of the central nervous system (by the electroencephalographic method) of the cardiovascular (according to the data of electrocardiography and the blood pressure) and respiratory systems in rabbits which had been injected with mitochondria from irradiated and healthy animals. These studies were made by A. B. Tsypin in M. N. Livanov's laboratory. In all, 17 rabbits were used in the experiments. A detailed description of the physiological studies is given in the article by N. N. Klemparskaya, L. I. Il'ina, R. V. Petrov and A. B. Tsypin published in 1959.

A comparison of the effects of different mitochondrial

preparations shows that the mitochondria of irradiated animals exert a greater toxic effect on the central nervous system, and preparations obtained from healthy rabbits exert a lesser effect. It should be pointed out that the phase of excitation of the central nervous system was considerably shorter in animals which received the mitochondrial extract from irradiated rabbits; the phase of depression in this case was, on the other hand, more pronounced. Changes in the cardiovascular system consisted of the fact that as early as 5-60 seconds after the injection of mitochondria from irradiated rabbits a change in the heart rate was noted in the recipient animals, whereby in nine rabbits a certain slowing of the heart rate occurred by 10-30 beats a minute. In four animals as well as in those which received the preparations from healthy rabbits the heart rate either was entirely unchanged, or a slight increase in its frequency was observed. The blood pressure under the influence of the mitochondrial preparation taken from irradiated rabbits began to drop one-three minutes after its administration and fell 40-50 millimeters of mercury. One-two hours after the injection the blood pressure recovered, approaching the original level. In cases which ended in death 3-48 hours after administration of the preparation, the fall in blood pressure which had been initiated progressed without showing any recovery period.

In animals which were given the mitochondrial extract from nonirradiated rabbits, administration of it either failed to change the pressure at all or caused a slight increase in it (by 10-20 millimeters of mercury). In these cases the blood pressure became normal as early as after 30-60 minutes.

The respiratory reaction to injection of the mitochondrial preparations was noted in all rabbits. In the majority of cases the mitochondrial injections led to an increased frequency of respirations if the respiration was slow and, conversely, injection of the preparations against the background of a fast respiratory rate caused a slowing of it. Injection of the mitochondria caused a slowing of the respiratory rate in 13 out of 17 rabbits which we observed and a quickening of it, in four. It should be pointed out that these respiratory rate changes were most pronounced where the animals were given the mitochondrial preparation obtained from irradiated rabbits. Death brought about by administration of the mitochondria was probably caused by paralysis of the nerve centers. This is confirmed by the fact that in the terminal period, first of all a loss of the electrical activity of the cerebral cortex occurs in the presence of impaired but still maintained activity of the cardiovascular and respiratory systems.

Table 50

Distribution of Labelled Preparations of the Mitochondria of Intestinal Mucosae of Irradiated and Healthy Rabbits in the Organs after Intravenous Injection (The Counter Background Was 14-18 Pulses per Minute)

| 1 Введен- ная фракция | 2 № кролика | 3 Доза препарата | | | 4 Радиоактивность 100 мг ткани, имп/мин | | | | | | | | | |
|--|----------------|------------------|---------------------------------|---|--|-------------|--------------|-----------------|------------------------------|-------------|--------------------|------------|--|--|
| | | 5 Объем, мл | 6 Активность тыс. имп/мин | 7 Биоло- гическая эффектив- ность | 8 Кровь | 9 Легкие | 10 Печень | 11 Селезенка | 12 Слизистая кишечника | 13 Почка | 14 Надпочечники | 15 Мозг | | |
| Раствор метио- нина 16 | 33 | 1,0 | 50 | Не смер- тельная 19 | 21 | 23 | 21 | 26 | 24 | 29 | 16 | 16 | | |
| Мито- хондрии нормаль- ного кролика 17 | 11 | 1,0 | 20 | Не смер- тельная 19 | 13 | 45 | 14 | 12 | 17 | 17 | 20 | 13 | | |
| | 36 | 2,0 | 100 | То же 20 | 32 | 64 | 18 | 18 | 15 | 15 | 16 | 17 | | |
| | 3 | 3,0 | 60 | Смер- тельная 21 | 15 | 129 | 17 | 13 | 17 | 20 | 15 | 14 | | |
| Мито- хондрии облу- ченного кролика 18 | 34 | 1,0 | 55 | Смер- тельная 21 | 27 | 73 | 29 | 24 | 18 | 24 | 15 | 14 | | |
| | 41 | 2,0 | 110 | То же 20 | 23 | 90 | 22 | 16 | 17 | 30 | 17 | 13 | | |
| | 43 | 0,3 | 18 | » 20 | 15 | 16 | 14 | 17 | 16 | 12 | 16 | | | |
| | 16 | 1,0 | 20 | » 20 | 16 | 19 | 11 | 15 | 11 | 11 | 13 | 12 | | |
| | 10 | 1,0 | 20 | » 20 | 28 | 22 | 13 | 17 | 16 | 14 | 17 | 13 | | |

1. injected fraction; 2. number of rabbits; 3. dose of preparation; 4. radioactivity of 100 milligrams of tissue, pulses per minute; 5. volume, cc; 6. activity, thousands of pulses per minute; 7. biological effectiveness; 8. blood; 9. lungs; 10. liver; 11. spleen; 12. intestinal mucosa; 13. kidneys; 14. suprarenal gland; 15. brain; 16. methionine solution; 17. mitochondria of normal rabbit; 18. mitochondria of irradiated rabbit; 19. not lethal; 20. the same; 21. lethal.

The facts presented constitute evidence, first of all, of the considerably greater (by 5-10 times) toxicity of intestinal tissues of irradiated animals than those of the controls and, secondly, of the

presence of the distinctive nature of their effect on healthy organisms; the developmental dynamics of the symptom complex in response to injection of the preparation from irradiated rabbits is different than in response to injection of control preparations.

The unique nature of the effect of the experimental preparations is illustrated also by experiments performed with labelled mitochondrial preparations and observations of the remote sequelae of the tissue injections (Table 50).

For the purpose of studying the characteristics of the effects of fractions from healthy and irradiated rabbits experiments were performed for determination of the distribution of preparations labelled with radioactive substance in various organs of rabbits (the rabbits were injected with methionine labelled for sulfur in a quantity of two millicuries per kilogram 18 hours before their organs were taken for examination).

The data obtained (Table 51) in all cases show fixation of the preparation label from the healthy rabbits chiefly in the lungs; in the other tissues the activity does not exceed the background. If labelled mitochondria from irradiated animals are injected, no selective accumulation of the label occurs in the lungs. In minimum lethal doses it is distributed uniformly throughout all tissues (rabbits 43, 16 and 10). After the administration of higher activities, the label accumulates in the lungs but is also found in the liver and kidneys (rabbits 34 and 41).

It has also been pointed out above that parenteral administration of liver homogenate or of preparations of liver cell microstructures as well as the amorphous part of the cell cytoplasm from the intestinal mucosa does not exert a direct toxic effect. However, such phenomena are not immaterial for the rabbits: injections of very small quantities of the preparations (10 milligrams of protein) lead to the development of a distinctive pathological process. The same effect is exerted by shock-producing preparations from the intestinal mucosa if they are not injected intravenously or are injected in non-lethal doses. This pathological state, described in 1956 by N. N. Klemparskaya, is characterized by the fact that 7-11 days after the injection a loss of weight and temperature elevation begin, and leukocytosis develops. After intradermal infection of these rabbits with the colon bacillus a considerably more intense inflammatory reaction develops than in control animals.

Along this line it is very important that the tissue preparations from irradiated rabbits exerted a great biological effect: a more pronounced inflammatory infiltrate developed with hemorrhages and necrosis. This was shown in the work of N. N. Klemparskaya, R. V. Petrov and L. I. Il'ina (1958). One of the experiment records is shown in Table 51.

Table 51

The Effect of Intravenous Injection of 10 Milligrams of Protein of Various Tissue Microstructures on the Course of the Local Inflammatory Reaction in Response to Intradermal Infection with 10^9 Colon Bacilli from a 24-Hour Culture

| ① Введенные препараты | | ⑦ От нормальных кроликов | | ⑩ От облученных кроликов | |
|------------------------|---------------|--------------------------|-------------------------|--------------------------|-------------------------|
| | | ⑧ № живот-ного | ⑨ реакция на зара-жение | ⑧ № живот-ного | ⑨ реакция на зара-жение |
| Печень ② | Цитоплазма ④ | 48 | + | 60 | +++ |
| | | 54 | + | 66 | +++ |
| | Митохондрии ⑤ | 51 | ++ | 65 | +++ |
| | | 55 | ++ | 68 | +++ |
| Слизистая ки-шечника ③ | Цитоплазма ④ | 56 | +++ | 61 | +++ |
| | | 57 | ++ | 62 | +++ |
| | Митохондрии ⑤ | 71 | ++ | 76 | +++ |
| | | 59 | + | 67 | +++ |
| Препараты не введены ⑥ | | 77 | ++ | 70 | +++ |
| | | 83 | + | — | — |
| | | 97 | ++ | — | — |

Key: +, infiltrate of less than three centimeters in diameter; ++, infiltrate from three to five centimeters in diameter; + + +, infiltrate larger than five centimeters in diameter; + + + +, the same plus hemorrhage and necrosis. 1, preparations injected; 2, liver; 3, intestinal mucosa; 4, cytoplasm; 5, mitochondria (*because of the shock-producing effect the preparation was injected intramuscularly rather than intravenously in this case); 6, preparations not injected; 7, from normal rabbits; 8, number of animals; 9, reaction to infection; 10, from irradiated rabbits.

2. Is Autoimmunization of an Irradiated Organism Possible?

The circulation of tissue proteins with altered biological, including antigenic, properties in the blood raises the question of the possible autoantigenic activity of these tissues in irradiated organisms. However, this problem runs up against the serious question of whether the realization of an autoimmunological response after irradiation is possible. This question has been discussed repeatedly at conferences in connection with the finding of antibodies against tissue proteins in the blood of irradiated animals (P. N. Kiselev and others, 1959; N. N. Klemparekaya, 1960; R. V. Petrov, 1960).

In contrast to a number of autoimmune diseases, where the appearance of autoantigens is not complicated by a pronounced inhibition of antibody production, in radiation sickness a rapidly developing disorder of the capacity of antibody production is observed. Therefore, in discussing the possibility of development of an immunological response to endogenous antigens after irradiation it is necessary to find out whether autoimmunization of the irradiated organism can occur. Actually, if an irradiated organism does not respond to injection of exogenous antigens with antibody production and if this normal function is affected practically immediately after irradiation, how can this organism provide for an immunological response to autoantigens?

N. N. Klemparekaya (1956, 1958) suggested that inhibition of antibody production after irradiation is the result of the reaction of the immunological system to a massive quantity of autoantigens coming from the tissues undergoing breakdown. As the result of this, according to the law of antigen competition, the "occupied" immunological system does not react to other antigens with antibody production. This suggestion is based on two basic observations. First of all, parenteral administration of extracts of homologous tissues, particularly intestine, to intact animals leads to the development of a pathological process, for which suppression of antibody production is typical. Secondly, administration of heterologous antigens before irradiation of the animals assures a mild course of the radiation sickness. In addition, the tissue antigens circulating in the irradiated organism must surely be "competitors" for the other antigens. All this indicates that the mechanism of antigen competition undoubtedly occurs in the irradiated organism. However, not all the facts known at the present time can be explained from this viewpoint. The basic facts are the following:

1. Protection of the spleen or smaller areas of lymphoid tissue, for example, the appendix or the bone marrow during irradiation is sufficient to leave the irradiated organism capable of reacting with antibody production to antigen injection. Jacobson and coauthors (1950, 1954), Smith and others (1958) showed that antibody titers in irradiated rabbits and mice remain at the normal figures if such protection is provided during irradiation. Subsequent removal of the shielded spleen deprives the animal of the ability of antibody production. A focus of lymphoid tissue uninjured by radiation provides for antibody production. If inhibition of antibody production after irradiation was the result only of the fact that the immunological system was "occupied" by a reaction to a massive dose of breakdown products of the animal's own tissues (for example, from the intestine), this focus of uninvolved lymphoid tissue would also be "occupied" and would not be able to give reactions to antigen injection.

2. This conclusion has been confirmed by facts of the recovery of the capacity of antibody production in cases where irradiated animals were injected with bone marrow cells or lymphoid tissues of a non-irradiated donor prior to immunization (Jacobson, 1954; La Via and others, 1958; Garver and others, 1959; Dixon and others, 1957; Makinodan and others, 1960). Makinodan and coauthors, for example, injected isologous splenic cells and antigen into lethally irradiated mice. It was determined that the more cells were injected the more antibodies were formed.

3. Non-irradiated lymphoid tissue cells are capable of producing antibodies on contact with antigen if they are implanted into irradiated animals (Harris, 1955; Dixon, 1957). If the cells are first irradiated (in vitro) they lose this capacity. Doses of 200-300 r markedly depress this function, and a dose of 500 r completely suppresses it. It is clear that in the case of irradiation in vitro the producer cells of the antibodies do not come into contact with the auto-antigens which circulate in the blood of the irradiated organism. Nevertheless, they lose the power of producing antibodies under the direct influence of irradiation.

According to the second suggestion, direct radiation injury to the cell and to its normal interaction with the internal medium of the organism is determinative in the disorder of antibody production; it leads to a change in the normal metabolic processes and processes of cell multiplication associated with a disorder of the highly specialized and sensitive function of immune globulin production.

This viewpoint is in agreement with the majority of experi-

mental data, including those which demonstrate the absence of a depressive effect of radiation on antibody production under the conditions where irradiation is performed after immunization. It is well known that prolongation of the inductive phase, which, depending on the times of immunization, can drag out to two weeks, rather than a simple reduction in the titer of antibodies produced is characteristic of radiation injury (Gengozian, Makinodan, 1958; R. V. Petrov, 1957; O. P. Peterson, I. A. Kozlova, 1958).

The inductive phase of antibody formation is distinguished by exceptional demands on metabolic conditions and sensitivity to various unfavorable factors. It cannot be carried out *in vitro*, under conditions of metabolic disorders and without morphologic differentiation of cells. This phase, of necessity, requires the existence of conditions which assure the possibility of normal division and differentiation of cells (J. Sternal, 1960). Ionizing radiation is one of the most powerful factors affecting metabolism, division processes and processes of cell transformation. Therefore, the inductive phase of antibody production cannot be accomplished in an irradiated organism. If the antigen is injected before irradiation, the inductive phase, which constitutes the organization of a highly specific mechanism of antibody production, goes on in the organism unaffected by radiation. Subsequent irradiation does not destroy this mechanism, just as it does not destroy many other enzyme systems (I. I. Ivano and others, 1956). Summing up, it may be said that there are two ideas which explain the inhibition of antibody production after irradiation. According to the first, the reason is the reaction of the immunological system to autoantigens; according to the second, the reason is a direct radiation injury of the cell and its normal interaction with the internal medium of the organism. If we accept the first idea, the answer to the question of the possibility of autoimmunization of the irradiated organism can be only in the affirmative. Moreover, the reaction of the organism to autoantigens is the reason for inhibition of antibody production to all other antigens. However, we must also analyze the second probability. Does it not constitute the basis for concluding that it is impossible for the irradiated organism to realize the autoantigenic stimulus? Not at all.

First of all, we should dwell on the problem of the nature of the radiation effect on antibody production (see Chapter 2). In this Chapter it was shown that the absolute suppression of antibody production is observed only in those cases where the animal dies in the first seven or, at most, 14 days. In all the other cases the effect of

radiation on antibody production is not characterized by absolute suppression of immune globulin production but rather by an increase in the inductive phase of this process; antibodies in determinable quantities appear later than normal. In this chapter it has also been shown that prolonged antigenic stimulation reduces the degree of inhibition of antibody production.

In Fig. 27 a schema is shown which compares several processes occurring in the body after irradiation. As an index of the inhibition of the power of producing antibodies a curve is shown on the Figure (Makinodan, Gengozian, 1958) characterizing the duration of the inductive phase of antibody production as a function of the time of injection of the antigen after irradiation. V. A. Sondak's (1957) data on the number of areas of micronecrosis in the bone marrow after irradiation of animals and our own data on redistribution of a protein label and on the dynamics of C-reactive protein in the blood are shown on the Figure as an index of tissue destruction and circulation of tissue antigens in the blood.

Comparing the indices presented on the schema it may be seen that tissue destruction and circulation of its products, that is, the autoantigenic stimulus, begins two-three hours after irradiation (as was shown in Section 1 of Chapter 7, the changes in the antigenic tissue properties begin to be recorded two-five hours after irradiation and, according to some data, even earlier), that is, during the period of maximum inhibition of the power of antibody production. However, the circulation of tissue antigens lasts at least two-three days. At this time, the power of responding to the antigenic stimulus becomes greater. While in the case of injection of antigen an hour after irradiation antibodies appear in the blood only on the 13th day, when the injection of antigen is made one-three days after irradiation they appear on the 11th-10th day. Considering the prolongation of the effect of autoantigens, which assures more effective antibody production by the irradiated organism, a more rapid appearance of auto-antibodies in the blood may be anticipated.

Based on this, it may be expected that autoantibodies in the early periods after irradiation may be found but only by exceedingly sensitive methods, as was confirmed by the experiments of N. N. Klemparskaya and N. V. Rayeva (1960). Beginning with the second-third week, antibodies can be demonstrated by ordinary immunological methods also. Sterzl (1960) showed that when methods of different degrees of sensitivity are used for finding small quantities of antibodies and for the detection of various antibodies the duration of the inductive phase is different.

The analysis presented makes it possible to answer the question put in the heading of this section in the affirmative. Autoimmunization of the irradiated organism with the appearance of autoantibodies is possible; however, in the initial periods very small quantities of autoantibodies in the blood must be expected.

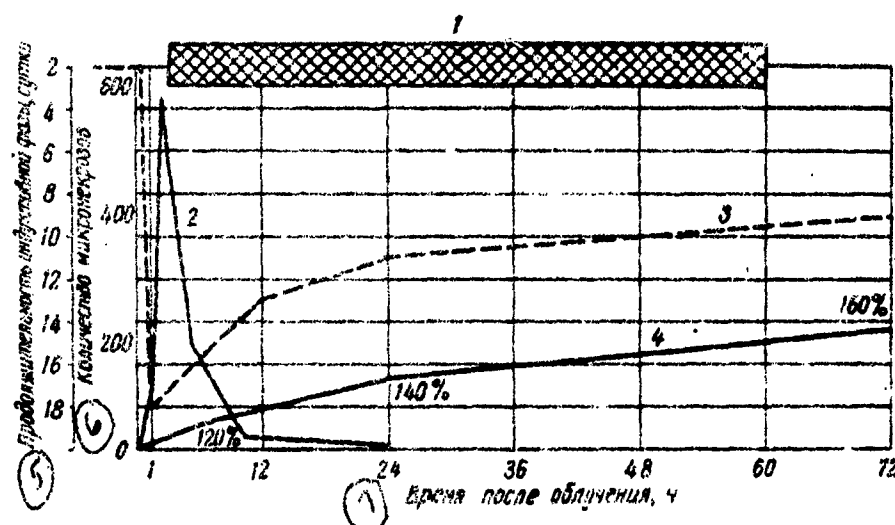


Fig. 27. Schema of Comparison of Times and Degree of Inhibition of Antibody Production, Occurrence of Areas of Micronecrosis in the Tissues, Appearance of C-Reactive Protein in the Blood and the Rate of Redistribution of the Protein Label After Irradiation: 1. CRP in the blood; 2. number of areas of micronecrosis per milligram of bone marrow (after V. A. Sondak); 3. duration of inductive phase of antibody production in days (after Makinodan and Gengozian); 4. redistribution of protein label (see Fig. 13); 5. duration of inductive phase, days; 6. number of areas of micronecrosis; 7. time after irradiation, hours.

3. Autoantibodies in the Blood of Irradiated Animals

Reports in the literature about autoantibodies in the blood of irradiated animals are few. A number of investigators (I. P. Mishchenko and M. M. Fomenko, 1934; P. N. Kiselev and V. A. Semina, 1959; V. A. Artamonova, 1959) used the complement-fixation test for detection of autoantibodies. However, the performance of the complement-fixation test with autologous tissue extracts, particularly under conditions of preliminary denaturative treatment of them, is associated with certain difficulties. First of all, there may be marked anticomplementary properties in these extracts. Secondly, the serum of some animals is capable normally of binding complement in the presence of autologous tissues, particularly liver, spleen and kidneys (Kidd, Friedewald, 1942). This reaction fails to occur only with extracts of skin and erythrocytes. It is very possible that the difficulty of performing the experiment has led to opposite results in two identical works: I. P. Mishchenko and M. M. Fomenko found complement-fixing autoantibodies in the blood of irradiated animals, and V. A. Artamonova did not. P. N. Kiselev and V. A. Semina anticipated these difficulties and obtained positive results. They used proteins denatured with alcohol, large doses of radiation and heating for the test. Therefore, they found antibodies against denatured homologous proteins rather than against the native autologous tissues, which are of the greatest interest.

By means of the precipitation test Yangisawa (1959) found autoantibodies in the blood of rabbits against the tissues of liver when half of this organ was subjected to focal irradiation. However, from the viewpoint of the discussion presented in the previous section, such data are not of great interest, because they were obtained after focal irradiation, that is, under conditions of minimum injury to the immunological system of the body. Experiments on the detection of autoantibodies by means of true autoantigens under conditions of whole body irradiation of animals have been presented by N. N. Klemparskaya in cooperation with N. V. Rayeva (1960) and by us in cooperation with G. M. L'vitsyna (R. V. Petrov and G. M. L'vitsyna, 1960). N. N. Klemparskaya and N. V. Rayeva used the highly sensitive method proposed by Hoigné for the study of allergic diseases in their experiments. The utilization of modern electronephelometers makes it possible to detect very small quantities

of antibodies, which are not detectable by other methods, with the use of this technique. Several species of animals were investigated which were irradiated with gamma- or x-rays in minimum lethal doses. Tissue extracts of individuals whose sera went into the autoantibody detection were used as antigens in the tests. Non-irradiated animals always gave a negative Hoigné test with autoantigens. The performance of the tests at various periods after irradiation gave a small number of positive results, beginning with the third day. Of 16 guinea pigs irradiated with a dose of 300 r, eight had positive reactions. Of 14 rabbits irradiated with a dose of 800 r, in five autoantibodies were detected. In the majority of cases the tests were positive when extracts of liver were used as the antigen; less often, when extracts of spleen or other organs were used.

In the experiments on dogs, in order to be able to carry out a dynamic study, a lysate of autologous erythrocytes was used as the antigen. Before irradiation there were no autoantibodies; they appeared after irradiation with a dose of 300 r of gamma rays (EGO-2 apparatus). On the third day after irradiation nine out of 16 dogs showed a positive Hoigné test; on the 10th day, eight out of 11; on the 20th day, six out of nine. In some of the animals the test was positive on the 45th day.

In recent years, mention has appeared in the literature of the fact that in a whole series of autoimmune diseases the production of incomplete antibodies is observed. Incomplete antibodies were found in the blood of parturient women in the cases of hemolytic disease of the newborn (Coombs, Mourant, Race, 1945; M. A. Umnova, 1954; T. G. Solov'yeva and N. S. Drobysheva, 1959 and others), hemolytic anemias and blood transfusions (Yu. I. Loriye, M. A. Umnova, L. I. Mikhaylova, 1956; I. Valevskaya, 1957; S. M. Martynov, Kh. V. Kuriy, Ya. I. Nikiferuk, A. R. Rabinovich, 1957; Ye. N. Mosyagina, 1958; Davidson, 1954 and others), agranulocytosis (A. S. Zverkova, 1957), thrombopenia (J. Dosset, 1959), hay fever (Feinberg, Davidson, Flick, 1956), and some other diseases. In the majority of cases incomplete antibodies were demonstrated by means of antiglobulin serum adsorbed on erythrocytes. However, recently, the existence of incomplete antibodies which react with other blood cells -- leukocytes (Killman, 1958; A. S. Zvertkova, 1957) and platelets (J. Dosset, 1959) -- has been proved.

The majority of these authors considers incomplete antibodies an index of autoimmunization of the organism. However, we have not

encountered any published studies directed at the detection of incomplete antibodies in radiation sickness. In connection with this, a determination was made of incomplete antibodies adsorbed on erythrocytes with the use of the direct and indirect Coombs method in three species of irradiated animals -- guinea pigs, dogs, and *Macacus rhesus* monkeys. One hundred and two guinea pigs, 45 dogs and 44 monkeys were repeatedly studied at various times (up to five years) after irradiation. The guinea pigs were irradiated with x-rays in a dose of 150 r (dose rate, 29.6-31.9 r per minute; current, 14 ma; voltage 180 kv; filter 0.5 mm Cu + 1 mm Al); dogs were irradiated with gamma-rays on EGO-2 apparatus (dose rate, 360-430 r per minute, dose of 300-350 r); monkeys, with doses of 300-700 r (gamma-rays with a dose rate of 70 r per minute). The experiments on monkeys were performed at Sukhumi in the Institute of Experimental Pathology and Therapy.

The Coombs test was performed with antiglobulin serum obtained by means of intravenous immunization of rabbits with serum globulins of guinea pigs, dogs and monkeys. Antiglobulin serum with a precipitin titer of no less than 1:5,000-1:6,000 was used. Before the utilization of antiglobulin serum in the test it was first inactivated and then adsorbed by erythrocytes of non-irradiated animals of the corresponding species until the erythrocyte-agglutinating activity had completely disappeared. In the tests use was made of fresh triply eluted erythrocytes of non-irradiated and irradiated animals in physiological saline solution (the dilution of erythrocytes after elution was 1:100). Blood was taken from guinea pigs by means of cardiac puncture; from dogs and monkeys, from the vein. The Coombs test was performed on a glass plate.

The direct Coombs test on the glass plate was accomplished in the following way: one drop of a two-percent suspension of eluted erythrocytes and one drop of adsorbed antiglobulin serum were applied to the glass plate. The drops were mixed. The test was read in 10 minutes.

Performance of the Indirect Coombs Test: Undiluted fresh triply eluted erythrocytes of non-irradiated animals in a volume of 0.1 cc were mixed in a test tube with the same volume of inactivated serum being tested. The mixture was incubated for 45 minutes at 37° C, and then the erythrocytes were eluted three times in an excess of physiological saline, and a two percent suspension was prepared. After this, one drop of the prepared suspension of sensitized erythrocytes and one drop of the antiglobulin

serum were applied to a glass slide. The test was read under the microscope in 10 minutes. Negative results were obtained with the erythrocytes of non-irradiated animals.

Positive Coombs tests in irradiated animals were not associated with the increased agglutinative property of erythrocytes, because serum from nonimmune rabbits did not in a single case produce agglutination of irradiated animal erythrocytes. Let us dwell on the results of the Coombs test obtained in each of the three species of animals separately.

Depending on the time that the test was performed, all the irradiated guinea pigs were divided into three groups (Table 52). In the guinea pigs of the first group the Coombs test was performed before irradiation and on the first, third, fifth, seventh, 10th and 14th days after irradiation.

Table 52

Results of the Direct Coombs Test in Guinea Pigs

| ① № группы | ② Количество свинков | ③ Доза облучения, р | ④ Срок после облучения, сутки | ⑤ Положительные реакции, % |
|---------------|----------------------------|---------------------------|--|----------------------------------|
| 1 | 32 | 150 | 1--14 | 3 |
| 2 | 45 | 150 | 15--30 | 51 |
| 3 | 18 | 150 | 40; | 0 |
| ⑥ Контроль | 40 | — | 3, 4, 6 месяцев — ⑦ | 1.5 |

1. number of group; 2. number of guinea pigs; 3. dose of radiation, r; 4. time after irradiation, days; 5. positive reactions, percent; 6. control; 7. months.

We were unable to find incomplete antibodies demonstrable by the Coombs test in irradiated guinea pigs for the first two weeks of acute radiation sickness. The majority of guinea pigs of the first group died at the end of the 14th-15th day as the result of radiation sickness and apparently also from loss of blood (cardiac puncture). Because we were unable to find incomplete antibodies adsorbed on the erythrocytes in the first two weeks of radiation sickness, the test

was performed in animals of the second group at later periods after irradiation, beginning with the 15th day after irradiation. This group included 15 animals from the first group. It was found that in 51 percent of the irradiated guinea pigs the direct Coombs test becomes positive on the 15th-20th-25th-30th day after irradiation. With subsequent observation at later periods after the irradiation (1.5-3-4-6 months) the positive direct Coombs test in the guinea pigs changed to negative. (Group III consists of surviving guinea pigs of Group II. The indirect Coombs test in the guinea pigs gave a negative result throughout the observation.

The data presented concerning incomplete antibodies demonstrable on erythrocytes with the Coombs test in irradiated guinea pigs permit us to draw the conclusion that incomplete antibodies in this species of animals appear two-four weeks after irradiation and disappear from the blood of animals six weeks after irradiation. The length of time the incomplete antibodies are maintained in the various guinea pigs is shown in Table 53.

The performance of the Coombs test with erythrocytes of irradiated dogs gave results similar to the previous ones. We made a study of the erythrocytes of two groups of animals: those irradiated with lethal doses of x-rays in a dose of 300-350 r and dogs which had had acute radiation sickness three years and eight months before.

The direct and indirect Coombs tests in irradiated dogs, just as in the case of guinea pigs, were negative in the first two weeks after irradiation. In a small number of irradiated dogs (4 out of 27) the direct Coombs test became positive three-five weeks after irradiation (Table 54). Thereby, in two dogs out of these four there was also a positive indirect Coombs test. During the period from 40 to 60 days after the irradiation incomplete antibodies were found in three out of nine dogs investigated. Of three dogs which showed a positive test at this time, the Coombs test was positive in two even in the third week after irradiation (two dogs with positive tests were dropped from the experiment for a number of reasons at this time); in one, the test had been negative until then.

The highest number of positive tests was demonstrated in animals which had been irradiated three years before (which were combined into Group II). Of the 18 dogs in this group the direct Coombs test was positive in 14. The indirect Coombs test in these dogs gave a negative result.

Table 53

Duration of Preservation of the Positive Coombs Test in
Irradiated Guinea Pigs

| ① №№ сукнин | ② Время после облучения в дозе 150 р, сутки | | | | | ① №№ сукнин | ② Время после облучения в дозе 150 р, сутки | | | | |
|----------------|--|-------|-------|-------|-----------------------|----------------|--|-------|-------|-------|-----------------------|
| | 1-14 | 15-20 | 21-25 | 26-30 | 40: 3. 4. 5 в мес. | | 1-14 | 15-20 | 21-25 | 26-30 | 40: 3. 4. 5 в мес. |
| 7 | + | + | напа | | | 56 | | + | + | + | напа |
| 3 | — | — | + | + | — | 58 | | + | + | + | — |
| 10 | — | — | + | + | — | 59 | | + | + | + | — |
| 11 | — | + | + | + | — | 60 | | — | + | + | — |
| 14 | — | — | — | + | — | 64 | | + | + | — | — |
| 20 | — | — | — | + | — | 70 | | — | + | + | — |
| 28 | — | — | + | + | напа | 81 | | — | + | + | — |
| 32 | — | + | + | + | напа | 84 | | — | — | + | — |
| 36 | — | + | — | + | напа | 85 | | + | + | + | — |
| 44 | — | + | + | + | — | 86 | | — | + | — | напа |
| 47 | — | — | + | + | — | 90 | | + | + | + | — |
| 53 | — | — | + | + | — | 95 | | — | — | + | напа |

1. No. of guinea pigs; 2. time after irradiation in dose of 150 r, days; 3. months; 4. died.

Confirmation of the data obtained concerning the occurrence of incomplete antibodies in the remote periods after irradiation was found from the examination of monkeys. The results of the experiments showed that in these animals (eight monkeys), irradiated with x-rays in a dose of 600 r, there are no incomplete antibodies demonstrable by the direct Coombs test in the first two weeks after irradiation. Prolonged observation of these monkeys was not carried out because of their deaths. For the purpose of detecting the production of incomplete antibodies in the remote periods after irradiation 36 monkeys were investigated which in the past had had acute radiation sickness as the result of the action of gamma- or x-rays in doses from 300 to 700 r (Table 55). Two monkeys were irradiated in 1954; nine, in 1955; eight, in 1956; four, in 1957; eight, in 1958; three, in 1959, and two in

Table 54

The Direct Coombs Test in Irradiated Dogs

| ① № группы | ② Количе- ство в группе | ③ Доза облуче- ния, р | ④ Резуль- тат реакции до облу- чения | ⑤ Результат реакции при постановке пробы после облучения | | | |
|------------------|----------------------------------|--------------------------------|---|---|--|---|-----------------------|
| | | | | ⑥ 1, 3, 5, 7, 14 сутки | ⑦ 20, 25 30-35 сутки | ⑧ 40-60 сутки | ⑨ 3 года 8 мес. |
| 1 | 42 | 300-350 | ⑩ Отрица- тельный | ⑪ Отрица- тельный | ⑫ Из 27 обследо- ванных у четы- рех + | ⑬ Из 9 обследо- ванных у трех + | |
| 2 | 18 | 300-700 | ⑭ Не об- следо- ваны | ⑮ Не обследо- ваны | ⑯ | | у 14 + ⑰ |

1. No of group; 2. No in the group; 3. dose of irradiation, r; 4. result of reaction before irradiation; 5. result of test when it was performed after irradiation; 6. days; 7. three years and eight months; 8. negative; 9. + in 4 out of 27 investigated; 10. + in three out of nine investigated; 11. not investigated; 12. +, in 14.

1960. It was found that in all the animals which had had acute radiation sickness in 1956-1958, the direct Coombs test was positive. A negative test was recorded only in seven animals which had had radiation sickness in 1955. In the majority of cases in the non-irradiated monkeys (25 out of 34) the Coombs test was negative. It is possible that a positive Coombs test in some of the non-irradiated monkeys is evidence of the presence of an undetected pathological process in them. This is particularly probable, since we selected monkeys from the general pack as control animals.

In Table 56 the results of the test are summarized depending on the times elapsing between irradiation and the investigation. A positive Coombs test was first recorded in one of the two monkeys a month after irradiation. During the period from five months to three years after irradiation incomplete antibodies were

found in 97 percent of the cases. After four years they were found in six out of 12 monkeys; after five years, in three out of nine. The impression is created that normalization with respect to this index begins four-five years after having had acute radiation sickness. This impression is confirmed in Table 57, in which the results of repeated examination of the monkeys in 1959 and 1960 are shown. Some of the animals, irradiated in 1956 and showing a positive test in 1959, gave a negative one in 1960.

In conclusion we should answer the following question: Have we the right, on the basis of a positive Coombs test in irradiated animals, to speak of the presence of incomplete autoantibodies in them? Erythrocyte agglutination in the presence of antiglobulin serum can depend not only on the presence of immune globulins possessing the properties of incomplete antibodies on the surfaces of the erythrocytes but also on the following factors:

1. A positive Coombs test can depend on an increased content of globulins in the blood of animals after irradiation. Increase in the quantity of globulin in the blood of irradiated animals is well known (I. I. Ivanov and others, 1956). However, it is impossible thereby to explain the positive Coombs tests, because the quantity of globulin in the blood increases as early as the first day after irradiation, while the Coombs test becomes positive only after two-three weeks and is recorded for several years, when the quantity of globulins is normal.

2. The positive test which we observed cannot be explained either by an increase in the nonspecific adsorptive activity of erythrocytes of irradiated animals, because increase in the adsorptive properties of tissues and proteins after the effect of ionizing radiation on the body is observed only during the first few hours or days, that is, when the Coombs test is usually negative (O. G. Alekseyeva, 1959; M. F. Fedotova, 1959; E. Ya. Grayevskiy and M. M. Korchak, 1959; our own data on the study of the anticomplementary properties of tissues).

3. It may also be supposed that positive Coombs tests in irradiated animals are associated with an increased nonspecific agglutinability of their erythrocytes in rabbit antiglobulin serum. However, specially performed control experiments showed that erythrocytes which agglutinate when antiglobulin serum is added do not agglutinate when normal rabbit serum is added.

Therefore, positive Coombs tests in irradiated animals can be explained by the presence of specifically bound globulins, that is, incomplete antibodies, on the surfaces of the erythrocytes. We are not inclined to believe that these are necessarily antierythrocyte antibodies. Having information about polyspecificity of incomplete

Table 55

The Direct Coombs Test in Macacus Rhesus Monkeys

| 1 Время облучения. год | 2 Время наблюдения | 3 Доза, р | 4 Облучение | | | | 5 Наблюдение | | |
|---------------------------------|--|--|---|-------------------------|----------|---|-------------------------|----------|---------------|
| | | | 5 Кличка | 6 Результаты реакции | | 7 Кличка | 8 Результаты реакции | | 9 Повторно |
| | | | | первично | повторно | | первично | повторно | |
| 1954 | 1960 1959 | 600 600 | Гриф Панама | ± | ± | Энаба Артан | — | — | — |
| 1955 | 1959 1959 1959 1959 1960 1960 1960 | 550 550 550 550 400 350 550 | Убон Табор Оша Чилан Эльс Шарат Кушка | + | + | Бювала Пресорка Плум Деринга Солонник Эполия Эптелия | — | — | — |
| 1956 | 1959 1959 1959 1959 1959 1959 1960 | 500 500 300+300 700 700 700 700 500 | Чур Хар Шуса Карлик Шафран Тюрко Цура Сабрак | + | + | Порт Маэри № 3262 № 3263 № 3270 № 3272 № 3279 № 3456 | — | — | — |

1. time of irradiation, year; 2. time of examination; 3. dose, r; 4. irradiated; 5. name of monkey; 6. results of test; 7. non-irradiated; 8. first time; 9. second time.

continuation of Table 55

| ① Эрсия облучения 1952 | ② Время облучения | ③ Доза, р | ④ Облучение | | | | ⑦ Наблюдение | | |
|---------------------------------|-------------------------|-----------------|-------------------|--------------------------|----------------|--------------|--------------------------|----------------|-------------|
| | | | ⑤ Клини- ка | ⑥ Результаты реак-ции | | Клини- ка | ⑧ Результаты реак-ции | | ⑨ Почерк |
| | | | | первичн- но | посторо- но | | первичн- но | посторо- но | |
| 1957 | 1959 | 300 | Ахмед | ± | | № 3281 | — | | |
| | 1959 | 300 | Бейта | + | | № 3254 | — | | |
| | 1959 | 700 | Алиас | + | ++ | № 3403 | — | | |
| | 1960 | 600 | Сенель | + | | № 3459 | — | | |
| 1958 | 1959 | 300 | Гондла | ± | | Эгуса | — | | — |
| | 1959 | 300 | Рейнур | ± | | Фухаря | — | | — |
| | 1959 | 300 | Мурин | ± | | Клима | + | ++ | + |
| | 1959 | 300 | Джунгар | + | | Нис | + | ++ | ++ |
| | 1959 | 300 | Дамбул | + | | Пат | + | ++ | ++ |
| | 1959 | 550 | Ган | + | ++ | Харис | + | ++ | ++ |
| | 1959 | 624 | Забран | + | ++ | Ева | + | ++ | ± |
| | 1959 | 624 | Дондас | ± | | Раджи | ± | | ± |
| ⑩ 1959 (ноябрь) | 1960 | 300 | Фан | + | | Герасим | + | | + |
| | 1960 | 300 | Фог | + | ± | Хорга | + | ++ | ++ |
| | 1960 | 300 | Фатус | + | + | Хариз | + | ++ | ++ |
| | 1960 | 315 | № 3436 | — | | | | | |
| 1960 | 1960 | 315 | № 3526 | — | | | | | |

Обозначения: — эритроциты не агглютинированы; ± единичные группы агглютинированных клеток, много свобод-
ных эритроцитов; + свободные эритроциты единичны; ++ все эритроциты агглютинированы.

10. November; 11. April

Key: - erythrocytes not agglutinated; ± occasional groups of agglutinated cells, many
free erythrocytes; +, scattered free erythrocytes; ++, all erythrocytes agglutinated.

Table 56

Direct Coombs Test in Monkeys Irradiated with Dose of 300-700 r

| Отношение ① | Контроль ② | Срок после облучения ③ | | | | | | | |
|-------------------------------|------------|------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| | | 1-15 сутки ④ | 1-15 сутки ⑤ | 1-15 сутки ⑥ | 1-15 сутки ⑦ | 1-15 сутки ⑧ | 1-15 сутки ⑨ | 1-15 сутки ⑩ | 1-15 сутки ⑪ |
| ⑦ Число положительных реакций | 9/34 | 0/8 | 1/2 | 3/3 | 8/8 | 7/8 | 8/8 | 6/12 | 3/9 |
| ⑧ Общее число случаев | | | | | | | | | |

1. ratio; 2. control; 3. time after irradiation; 4. days; 5. month (s); 6. year (s); 7. number of positive tests; 8. total number of cases.

autoantibodies (Valevskaya, 1957; Loriye and others, 1956; Martynov and others, 1957), it seems to us very probable that erythrocytes, as the main mass of blood cells, react with autoantibodies entering the blood stream and are, therefore, only unique "carriers" of them. The autoantigenic stimulus for their production may not come from erythrocytes but rather from other tissues injured by radiation. However, for the purpose of clarifying this additional experiments are required with the use not only of erythrocytes but also other cells.

4. Demonstration of the State of Sensitization to Tissue Antigens

The second immunological phenomenon which may be observed as the result of the appearance of autoantigens in the body is associated with the possibility of development of a state of sensitization. It should be kept in mind that this state can occur in a manner which is far from being parallel with antibody buildup (A. T. Kravchenko and N. V. Galanova, 1948); a number of phenomena of cell sensitization developing without them are well known. As recent works have shown in the field of acquired tolerance, the specific cellular reactivity is created earlier in the

Table 57

Results of Two Examinations of Irradiated Monkeys for the Presence of Incomplete Antibodies in the Blood

| Кличка ① | Время облуче- ния, год ② | Доза, р ③ | Результаты обследования ④ | |
|---|--------------------------------|---|--|--|
| | | | 1959 г. | 1960 г. |
| Табор Оша Убон | 1955 | 550 550 550 | -- -- + | -- -- + |
| Чур Карлик Тюрко Хар Шуса Шафран Цура | 1956 | 550 700 700 500 300+300 700 700 | ++ ++ ++ ++ ++ ++ ++ | -- -- -- -- ++ ++ ++ |
| Райнур Мурян Джунгар Ган Дондас | 1958 | 300 300 300 300 624 | ± ++ ++ ++ ± | ++ ++ ++ ++ -- |

1. name of monkey; 2. time of irradiation, year; 3. dose, r; 4. results of the study.

course of ontogeny than the power of antibody production (Sterzl, 1960). All this permits us to suppose that in the irradiated organism the development of sensitization in response to tissue antigens is possible not only in connection with the appearance of autoantibodies but also prior to their production.

The effectiveness of desensitization therapy in radiation sickness and numerous indirect data illustrating the analogy between a number of the manifestations of radiation injury and states associated with sensitization have been presented in our monograph

(N. N. Klemparskaya, O. G. Alekseyeva, R. V. Petrov, V. F. Sosova, 1958). However, these indirect data, based on analogies, could not satisfy us, and N. N. Klemparskaya for a number of years has sought means of directly proving the existence of sensitization of irradiated animals to tissue antigens.

In 1958, at the Second International Conference on Peaceful Uses of Atomic Energy in Geneva, N. N. Klemparskaya in cooperation with V. V. Shikhodyrov presented such proof, obtained from administration of antigens by the Freund and Stone method. The experiments were performed on white mice irradiated with a dose of 600 r and on guinea pigs irradiated with a dose of 500 r. On the third-seventh day after irradiation antigens were injected into the animals in the skin of the upper lip; antigens were used, to which it was possible to expect the existence of sensitization. As food proteins milk was used; as microbial antigens, a suspension of a 24-hour colon bacillus culture (100,000,000 cells in one cc of physiological saline). In these experiments extracts of homologous tissues of liver, spleen, bone marrow and small intestine of healthy and particularly irradiated animals were of the greatest interest. A tissue heteroantigen was also used -- horse serum. It was determined that the hyperergic reaction does not develop in response to the injection of heterogenous or microbial antigens. Extracts of homologous tissues (liver, spleen, bone marrow) of healthy and irradiated animals are also inactive. Only the injection of small intestinal extracts of irradiated animals of the same species on the third-seventh day after irradiation causes the development of a local hyperergic reaction, characterized by edema, tissue necrosis and hemorrhages. The edema spreads over the animal's entire head. Tissue extracts of the small intestine of healthy animals caused a much less pronounced reaction. Thus, using a four-plus system of reading the reaction, out of 58 irradiated mice which received a preparation from the intestinal wall of irradiated animals on the third day, 13 gave a + + + + test; 11, + + +; 5, + +; and 14, +. Of 34 animals which received the intestinal preparation from normal mice, no sensitization phenomena were observed at all in 24; only two gave + + + test; five, a + + test, and three, a + test.

These experiments clearly illustrate the development of the state of sensitization after irradiation with respect to antigens of intestinal tissue pathologically altered as the result of irradiation.

We, in cooperation with V. V. Shikhodyrov and M. F. Sbitneva,

found signs of sensitization of the areolar connective tissue in acute radiation sickness (V. V. Shikhodyrov, R. V. Petrov, M. F. Shitneva, 1958). Thereby, the intestinal tissue antigens altered under the influence of irradiation also proved to be most responsible for the phenomena which we had observed. In 1954-1957 study of the reaction of areolar connective tissue in acute radiation sickness showed that some changes occurring in the fibroblasts and macrophages have much in common with the changes occurring in sensitization (V. V. Shikhodyrov, 1954; N. A. Kravetskiy, 1957). For the purpose of elucidating the nature of the sensitizing agent the method of studying the pathological changes in the tissues under the influence of various sensitizing factors and drawing morphological parallels between these changes and the changes observed in radiation sickness was selected. The studies were made on 280 white mice weighing 22-24 grams. The experiments included seven groups of animals.

In the first group a study was made of the reaction of areolar connective tissue to whole body irradiation. The animals were irradiated on an RUM-3 x-ray apparatus under the following conditions: 180 kv, 15 ma, filters of 0.5 mm Cu + 1.0 mm Al; FSD, 56 centimeters; dose rate, 19.2 r per minute; irradiation time, 26 minutes; total dose, 500 r.

In the second group a study was made of the areolar connective tissue reaction to the injection of heterogenous rabbit serum. Fourteen days after the first injection, 0.5 cc of serum was injected intraperitoneally a second time. As the result of the reacting injection, the mice developed signs of anaphylactic shock, and part of the animals died.

In the third group of experiments a study was made of the reaction of areolar connective tissue after sensitization of mice with the organs of irradiated animals of the same species. For this purpose, 10 mice were irradiated on an EGO-2 apparatus with a dose of 800 r; dose rate, 500 r per minute; and they were killed on the third day. From the liver, kidneys, spleen and intestine a suspension was prepared the extract of which was injected into the animals. After 14 days, a similarly prepared extract was injected again. In contrast to the previous series, signs of fatal anaphylactic shock did not develop in the animals. In the fourth group of experiments a study was made of the areolar connective tissue reaction after the animals had been injected with an extract of

intestinal mucosa of intact animals. In the fifth group, extract from the tissue of the intestinal mucosa from mice which had been killed three days after irradiation with a dose of 800 r was used for the injection. In the sixth and seventh groups a study was made of the reaction of areolar connective tissue to the injection of splenic extracts of intact animals and animals killed 15 hours after irradiation with a dose of 800 r, that is, during the period of the most pronounced destructive changes in the lymphoid apparatus.

In all groups, mice were decapitated 2, 6, 24 hours and 2, 3, 5, 7, 10, 15 and 20 days after the first and second antigen injections. From the subcutaneous tissue of the back a series of film preparations was made which was fixed in 10 percent neutral formalin solution and stained with iron hematoxylin by the Yasvoin method, with picrofuchsin and toluidine blue.

For the purpose of judging the qualitative changes in the elements of areolar connective tissue, a count was made of the cells according to a certain schema. As a control, preparations of areolar connective tissue of healthy mice were used, the cell composition of which consisted of pericytes and mast cells (one-seven percent), fibroblasts (the total number amounted to 78-58 percent), of which young forms amounted to 6-14 percent; mature forms, 24-53 percent; old forms, 15-11 percent; degenerating forms, two-nine percent, and naked nuclei, 1.5-0 percent; the total number of macrophages varied within limits of 17.5-26 percent; they consisted of cells with rounded cytoplasm (16-22 percent), with vacuoles and pseudopods (1.5-3 percent) and degenerating forms (0-1 percent). Pseudo-neutrophilic leukocytes amounted to 4.5-8 percent. Because the main mass of mast cells and pericytes are located along the courses of the blood vessels, in making the differential count consideration was given only to the tissue pericytes and mast cells, that is, part of these cells.

Simultaneously, a study was made of blood. The quantity of hemoglobin, red blood count, reticulocyte count, white blood count, differential count and sedimentation rate were determined. Consideration was also given to cytologic changes in the blood cells. After a single irradiation of white mice with x-rays in a dose of 500 r deep-seated degenerative changes appeared in the areolar connective tissue which were of a phasic nature.

Beginning with the first few hours after irradiation the development of two parallel processes was observed. First of all,

activation of the areolar connective tissue occurred which was associated with active maturation of pericytes into young fibroblasts and macrophages, the number of which increased. Secondly, accelerated postmaturation changes of elements of the fibroblastic series occurred with the appearance of a large number of old and degenerating fibroblasts and then of naked nuclei. In the first two days after irradiation a unique "foaming" of the cell cytoplasm was observed. In the fibroblasts vacuoles of quite large size appeared which broke through the cytoplasm. After the appearance of vacuoles and pseudopods in the cytoplasm of macrophages the cells broke down. There was a considerable reduction in the number of pseudo-neutrophilic leukocytes. After the changes occurring in their cytoplasm the mast cells also degenerated. At the climax of acute radiation sickness deep-seated degenerative changes were noted in the cellular-fibrillar elements of the areolar connective tissue. However, during the recovery period, which began after 10-15 days, regeneration of the cellular elements was observed with the characteristic phenomenon of a regenerative shift to the left in the differential count of the areolar connective tissue characteristic of this. The blood changes were typical of acute radiation sickness. This series of experiments served as a standard, with which the results of all subsequent experiments were compared.

In experiments with the injection of heterogenous serum a pronounced sensitization reaction of the animals was observed with characteristic changes in the areolar connective tissue. These changes consisted of accelerated maturation of fibroblasts and the occurrence of a large number of young cells. Most characteristic of this reaction was the appearance of a large number of mature cells. Two-six hours after injection of serum the appearance of a large number of small granules was noted in the cytoplasm, chiefly the endoplasm of the cells. A unique "foaming" of the cytoplasm of the fibroblasts occurred. Somewhat later, after 6-24 hours, vacuoles appeared in the cytoplasm of mature fibroblasts which increased in size, assuming the appearance of large cavities located in the cell endoplasm. During this period there was an increase in the number of macrophages to 46 percent. The cells were arranged in small groups or chains. There was also an increase in the number of mast cells. After five-eight days a "juvenescence" of the cell composition of the areolar connective tissue was observed with subsequent normalization of the number of

macrophages and other cells.

With a second injection of the reacting dose of serum death of some of the animals was noted which ensued in the first hour with signs of anaphylactic shock. Desensitization in the surviving animals led to a loss of the changes specific of sensitization (macrophagocytosis and vacuolization of the cytoplasm of mature fibroblasts).

Therefore, a single injection of heterogenous serum causes changes in the areolar connective tissue similar to changes in this tissue during the first few hours after irradiation (vacuolization the fibroblast cytoplasm, and macrophagocytosis /increase in the number of macrophages/); however, the subsequent and most essential disorders developing in radiation sickness have an entirely different trend and cannot be compared with sensitization by heterogenous serum.

Of the greatest interest, it seems to us, are experiments in which extracts of organs of irradiated animals are injected (intestine, liver, spleen). In the first day after the injection, an accelerated maturation of areolar connective tissue cells occurred and there was an increase in the number of mature fibroblasts. In the cytoplasm of these cells a large number of vacuoles appeared. Characteristic of the areolar connective tissue reaction to the injection of tissues of irradiated animals was also a considerable increase in the macrophage count (256 percent). The cells were arranged in the form of small groups of chains. In some cells the cytoplasm was foamy, contained small vacuoles. An increase was noted in the number of pseudoneutrophilic leukocytes. On subsequent days the proliferation phenomena were replaced by a pronounced degeneration of a number of cells, chiefly macrophages and leukocytes, as the result of which normalization of the cell composition occurred.

After a second injection of the tissues of irradiated animals changes occurred as early as after two hours, which largely resembled the areolar connective tissue reaction at the climax of acute radiation sickness. In the fibroblastic series of cells there was an increase in the number of degenerating forms and naked nuclei. A degenerative shift to the right was noted. The major part of the macrophages as well as leukocytes degenerated. More severe degenerative changes occurred after six hours, when, along with the increase in the number of old degenerating fibroblasts and naked nuclei, there was a reduction and in some cases a complete

disappearance of tissue pericytes and young fibroblasts. There was a reduction in the number of macrophages; among these cells degenerating forms predominated. Tissue mast cells contained a small number of secretion granules. Further observation showed that during the first eight days changes of a degenerative nature occur in the areolar connective tissue. The reduction in the number of macrophages, and mast cells continues; fibroblasts degenerate. Only beginning with the 8th-10th day do signs of regeneration of the cellular composition of the areolar connective tissue occur. Around the blood vessels many pericytes accumulate; there is an increase in the number of young fibroblasts and macrophages. Therefore, a second injection of extracts of the organs of irradiated animals leads to a change in the areolar connective tissue, which resembles its reaction at the climax of acute radiation sickness. After a single injection of intestinal mucosal extract of healthy mice no considerable changes could be found in the areolar connective tissue. At the same time, a second injection caused a pronounced activation of this tissue, which was associated with a juvenescence of the cell composition and an increase in the total number of macrophages. Simultaneously, a slight vacuolization of the fibroblast cytoplasm occurred.

In experiments with the injection of an extract of intestinal mucosa of irradiated animals a reaction was observed which was different from that observed in the previous series. After the first injection changes in the areolar connective tissue were poorly expressed, and the cell composition deviated only slightly from the normal.

After repeated injection of the extract from the intestine of irradiated animals pronounced degenerative changes occurred in the cellular elements of the areolar connective tissue. The earliest and most severe change occurred in the macrophages, the total number of which decreased as the result of pronounced degeneration. The fibroblast reaction to the injection was less distinct and was manifested in a reduction in the number of mature and an increase in the number of old and degenerating fibroblasts. The changes lasted a short time. After two-three days regeneration began, and by the 15th-20th day complete restoration of the cell composition of the areolar connective tissue occurred.

Therefore, an injection of intestinal tissue extracts of an irradiated animal depresses the areolar connective tissue with the

greatest changes in the macrophages (degeneration, reduction in their total number) and causes increased maturation of fibroblasts. These changes also have much in common with those observed in the areolar connective tissue in acute radiation sickness.

The injection of splenic tissue extracts from intact mice did not cause any appreciable reaction of the cellular elements of the areolar connective tissue. At the same time, a splenic extract of irradiated mice appreciably activated the areolar connective tissue, which was associated with a juvenescence of the cells of the fibroblastic series and an increase in the number of macrophages. Vacuolization of the fibroblasts in these experiments could not be observed.

These experiments make it possible to express a number of principles with regard to the changes in areolar connective tissue in acute radiation sickness. As we have already pointed out, the reaction of areolar connective tissue to irradiation is of a certain phasic nature. One of the reasons for this reaction, in our opinion, is sensitization of the body with tissue breakdown products arising after irradiation. The entrance of breakdown products of intestinal mucosa, blood cells, etc. into the blood stream causes an early reaction of the areolar connective tissue which is manifested in the form of activation of it (increase in the number of macrophages, juvenescence of the cell composition, foaming and vacuolization of the fibroblast cytoplasm). The lack of specificity of this reaction is quite obvious, because we obtained similar changes after the injection of heterogenous serum. Therefore, the activation of areolar connective tissue occurring after irradiation as well as some changes in it may be a reflection of sensitization of the organism. However, the most essential changes in the areolar connective tissue in response to irradiation consists of destruction of its cellular elements. In this respect, experiments with repeated injection of various antigens give interesting results. While in experiments the repeated injection of heterogenous serum normalizes the areolar connective tissue, in experiments with repeated injection of organs of irradiated animals and, particularly, intestine, deep-seated changes occur in the areolar connective tissue of a degenerative nature. The first and second injections of tissues from normal animals (spleen, intestinal mucosa) do not lead to the development of changes in the areolar connective tissue like the reaction of it to irradiation.

Therefore, the connective tissue reaction in acute radiation sickness to a certain degree may be related to the development of the process of sensitization by tissue antigens altered under the influence of irradiation.

Summing up the material of this chapter, first of all we should emphasize the fact that tissue preparations from irradiated animals are several times more toxic when injected intravenously into animals of the same species than preparations made from normal tissues. The greatest toxicity is shown by microsomes of the intestinal mucosa. The minimum lethal dose for the rabbit is equal to one milligram of protein if the microsomes were taken from an irradiated animal and 11 milligrams, from an intact animal. It should be noted that the more pronounced toxicity of tissues obtained from irradiated animals is illustrated not only by their lower lethal doses but also by finer indices, detected by electrophysiological methods (the studies of A. B. Tsypin) and by means of determination of sensitivity to infection in animals which had been given non-lethal doses of active tissue preparations.

An analysis of the nature and times of injury to antibody production after irradiation shows the possibility of realization of the autoantigenic stimulus by the irradiated organism. Direct experiments on the detection of autoantibodies in the blood of irradiated animals have confirmed this. By the Coombs methods incomplete autoantibodies were found in the blood of monkeys, dogs and guinea pigs in the second-third week after irradiation. The length of time the autoantibodies were found is measured in months. When other methods are used autoantibodies are found earlier after irradiation (P. N. Kiselev, N. N. Klemparskaya). The experiments of N. N. Klemparskaya and V. V. Shikhodyrov prove the existence of a state of sensitization to homologous tissues in acute radiation sickness. In our experiments, performed in cooperation with V. V. Shikhodyrov and M. F. Shitneva, it was shown that morphological changes in the areolar connective tissue in acute radiation sickness can be related to the development of autosensitization.

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Chapter VIII

STUDY OF THE EFFECT OF IMMUNE SERA AGAINST TISSUES OF IRRADIATED ANIMALS

The most convincing proof of the pathogenic role of tissue antigens in radiation sickness is the attempt to affect them specifically and immunologically. The first idea which occurs thereby is the idea of attempting to immunize animals before irradiation with tissue antigens of irradiated animals. However, this idea immediately encounters serious objections, which are based on the following. First of all, parenteral administration of homologous tissues to animals is far from being a harmless procedure. As has been shown in the previous chapter, such injections contribute to the development of a unique pathological process which in a number of cases terminates fatally. Secondly, the concentration of altered antigens is very low by comparison with the normal antigens in the tissues of irradiated animals (immunizing material). Such immunization would be more encouraging if we knew the nature of the "radiation antigens" and could immunize animals with pure preparations of them. Thirdly, the sensitizing significance of altered tissue antigens may be greater than of simple immunizatory significance. In this case we cannot expect a favorable effect from immunization with tissues of irradiated animals either.

Experiments performed in combination with L. I. Il'ina confirm what has been stated -- we were unable to obtain positive results by immunization of rats with tissue preparations of irradiated rats. In Table 58 the results of this experiment are shown.

The group of animals was irradiated with gamma-rays on an BGO-2 apparatus with a dose of 5,000 r and a dose of 470 r per minute. Twenty-four hours after this, the normal and irradiated rats were killed, and from the liver tissues preparations of cell nuclei, mitochondria, microsomes and hyaloplasm were isolated according to the method described above. After determination of the quantity of nitrogen, suspensions of the preparations were prepared in physiological saline solution containing 4.5 milligram per cc. For two days straight the healthy rats were given one cc of one tissue preparation or another from irradiated or normal animals intra-

Table 58

The Survival Rate of Rats Which Prior to Irradiation Received Tissue Preparations of Normal (H) Rats or Rats Irradiated (O) with a Dose of 5000 r

| Ткань, использованная для иммунизации (1) | Число животных в группе (2) | Доза облучения, р (3) | Число погибших (4) | Ткань, использованная для иммунизации (1) | Число животных в группе (2) | Доза облучения, р (3) | Число погибших (4) |
|---|-----------------------------|-----------------------|--------------------|---|-----------------------------|-----------------------|--------------------|
| Н-ядра (5) | 10 | 750 | 9 | О микросомы (8) | 9 | 750 | 8 |
| О " (6) | 10 | 750 | 9 | Н гиалоплазма (9) | 9 | 750 | 8 |
| Н митохондрии | 10 | 750 | 8 | О " (9) | 10 | 750 | 9 |
| О " (7) | 9 | 750 | 8 | — | 10 | 750 | 10 |
| Н микросомы (7) | 10 | 750 | 8 | — | | | |

1. tissue used for immunization; 2. number of animals in the group; 3. radiation dose, r; 4. number of those which died; 5. H-nuclei; 6. H-mitochondria; 7. H-microsomes; 8. O-microsomes; 9. H-hyaloplasm

muscularly. On the third day, they received two cc of the suspension, and on the sixth day all the rats were irradiated with a dose of 750 r. The results of the experiments showed the absence of any pronounced protective effect of such immunization. This is seen also from a comparison with the control group of animals and with those which received tissues preparations of normal rats. In cooperation with N. N. Klemparskaya and L. I. Il'ina a large number of such experiments was performed on rabbits with the use of different tissues, different routes and plans of injection (N. N. Klemparskaya, R. V. Petrov, L. I. Il'ina, 1958). In a number of experiments, with the use of injection schemata from which a desensitizing effect of tissue preparations might be expected favorable results were obtained. However, the number of animals does not permit us to speak of the statistical significance of these data. V. A. Artamonova (1959) was also unable to "immunize" against irradiation. For the "immunization" she used extracts of the livers of irradiated animals.

In connection with this, it was decided to go about acting immunologically on the tissue antigens occurring after irradiation by a different method -- by means of using antisera obtained from the immunization of rabbits or goats with the tissues of irradiated animals of a different species.

It is well known that tissue antisera possess marked cytotoxic effects which are destructive to the same species of animal. Sera obtained from immunization of irradiated animals with tissues must certainly possess such an effect and exert an aggravating effect on the course of radiation sickness. However, adsorption of them by normal tissues changes their biological effect.

1. Aggravating Effect of Whole Sera

In the literature there are a number of data on the effect of cytotoxic sera on the course of radiation sickness in animals. In these works use was made of antisera obtained from immunization with tissues of normal animals. The results of published experiments are identical. Thus, M. F. Sbitneva (1956), studying the effect of myelotoxic and antireticular sera in experiments on mice and dogs, showed that high doses of them markedly aggravate the course of radiation sickness. This is recorded also from the survival rate of the animals and from hematological and other clinical indices. The use of low, so-called stimulating doses of cytotoxic sera leads to a somewhat more favorable course of radiation injury. These data are in complete agreement with the experiments of Z. D. Zekhova (1958), S. A. Korol' and L. A. Umanskiy (1958) as well as with the experiments of foreign investigators (Benko, 1953; Loiseleur and others, 1959).

For our further discussion it is very important to emphasize the size of the stimulating, that is, exerting a favorable effect, dose of cytotoxins. In experiments on mice, this dose was 0.00001 cc for antireticular and myelocytotoxic sera (Z. D. Zekhova, 1958; M. F. Sbitneva, 1956); for rats, 0.0001 cc with antisera obtained from immunization of goats or rabbits with the tissues of irradiated animals. The rabbits were immunized with the tissues of white rats; goats, with rabbit tissues.

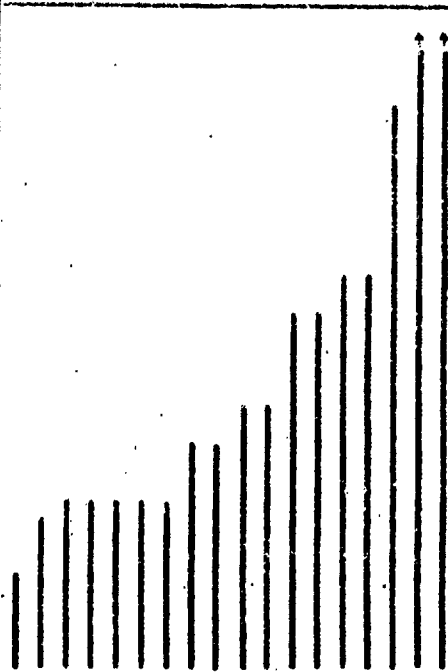
The plan of immunization of the rabbits was the following: a 10 percent tissue homogenate obtained from homogenization in a high speed blender was centrifuged at a rate of 2500 revolutions per

minute for 10 minutes. This provided for the settling of intact cells and fibers. The supernatant fluid was injected five times intravenously at four-day intervals in increasing doses -- 0.5, 1.0, 1.5, 2.0 and 2.0 cc. For each immunization a fresh preparation was made. All the procedures for the preparation were carried out on ice. Blood was taken from the rabbits on the eighth-tenth day after the last antigen injection. The antibody titers were determined in the complement-fixation test. If the immunization was carried out with serum, it was used undiluted, and the antibody titer was determined by the precipitin test. In the case of immunization of goats the antigen was injected in three courses with four-day intervals between them. Each course lasted three days, with the daily injection of antigen on the first day subcutaneously; on the second and third days, intravenously. The doses for the first course were 1.0, 2.0 and 2.0 cc; for the second and third courses, 4.0, 4.0 and 4.0 cc. Blood was taken on the ninth day after the last injection. Sera obtained from immunization with the tissues of irradiated animals (in all experiments tissues of animals killed two days after irradiation on an EGO-2 apparatus with a dose of 2000 r were used; all the animals injected with immune sera were irradiated on an EGO-2 apparatus at a dose rate of 431-470 r per minute), in the same way as ordinary cytotoxic sera, exert an aggravating effect on the course of radiation sickness, once again confirming the data in the literature.

In Table 59 the results of the experiment are presented, demonstrating the effect of the intramuscular injections of whole rabbit antisera, obtained from immunization with the tissues of irradiated rats, on the survival rate of rats irradiated with a dose of 650 r. The sera were injected three times intramuscularly. The first injection was given 18 hours before irradiation; the second, in the first 15 minutes after irradiation, and the third, after six hours. For the purpose of characterizing the immune sera their titers with respect to certain tissues are shown in the Table. The antibody titers were determined in the complement-fixation test (for the description see above). From the Table the increase in the mortality rate and considerable reduction in the average lifespans of the irradiated animals which received the injection of tissue antisera are seen. Similar results were obtained in experiments on rabbits, which were injected three times with antisera obtained from immunization of goats with the tissues of irradiated rabbits (Table 60).

Table 59

The Effect of Whole Rabbit Antisera Obtained from Immunization with Tissues of Irradiated Rats on the Survival Rate of Rats Irradiated with a Dose of 650 r

| ① Введенная сыворотка | ② Лечебная доза | ③ Доза | ④ Доза | ⑤ Титр сыворотки по отношению к различным тканям | ⑥ Продолжительность жизни, сутки | | | | | | | ⑦ Поло | ⑧ Время | ⑨ Средняя продолжительность жизни |
|--------------------------------|--------------------|-----------|-----------|---|---|---|----|----|----|----|----|-----------|------------|--------------------------------------|
| | | | | | 2 | 6 | 10 | 14 | 18 | 22 | 26 | 30 | | |
| Нормальный кролик (контроль) ⑩ | 0.4 | 0.5 | 0.3 | — |  | | | | | | | 16 | 2 | 11.5 |
| Анти-О сыворотка № 722 ⑪ | 0.2 | 0.5 | 0.3 | Кишечник О 1 : 80 | | | | | | | | 7 | 0 | 7.0 |
| | | | ⑫ | Кишечник Н 1 : 40 | | | | | | | | | | |
| | | | ⑬ | Печень Н 1 : 640 | | | | | | | | | | |
| | | | ⑭ | Печень О 1 : 640 | | | | | | | | | | |
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⑪ injected serum; ⑫. second injection; ⑬. third injection; ⑭. serum titer against various tissues; ⑮. lifespan, days; ⑯. died; ⑰. average lifespan, days; ⑱. of normal rabbit (control); ⑲. anti-O liver No 722; ⑳. intestine-O; ㉑. intestine H;

continuation of Table 59

| 1 | 2 | 3 | 4 | 5 | 6 Продолжительность жизни, суток | | | | | | | | 7 | 8 | 9 |
|--------------------------|-----|-----|-----|--|--|---|----|----|----|----|----|----|---|---|---|
| | | | | | 2 | 6 | 10 | 14 | 18 | 22 | 26 | 30 | | | |
| Вакцина № 984 | 0,2 | 0,5 | 0,3 | Титр сыворотки по отношению к разведенным вакцинам | (12) О-кишечник 1:160 Н-кишечник 1:80 Н-печень 1:40 О-печень 1:80 | | | | | | | | | | |
| | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | |
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| | | | | | | | | | | | | | | | |
| Анти-О сыворотка № 69 | 0,2 | 0,5 | 0,3 | (16) О-сыворотка 1:1000 О-кишечник 1:50 Н-кишечник 1:10 Н-печень 1:10 О-печень 1:20 | | | | | | | | | | | |
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| | | | | | | | | | | | | | | | |

* dose in cc. / continued from previous page 14. liver H; 15. liver O; 16. O-serum; 17. anti-O intestine No 984; 18. anti-O serum No 69

Table 60

The Effect of Whole Goat Antisera Obtained from Immunization with Tissues of Irradiated Rabbits on the Survival Rate of Rabbits Irradiated with a Dose of 800 r

| 1 Введенная сыворотка | 2 Первое введение | 3 Второе введение | 4 Третье введение | 5 Истор сыво- ротки по от- ношению к различным типам | 6 Продолжительность жизни, сутки | | | | | | | 7 Плюс | 8 Вс- его живот |
|---------------------------------|-------------------------|-------------------------|-------------------------|---|--|---|----|----|----|----|----|-----------|--------------------------|
| | | | | | 2 | 6 | 10 | 14 | 18 | 22 | 26 | | |
| Нормальной козы (контроль) ① | 5.0 | 5.0 | 2.0 | — | —< | | | | | | | | |

* dose in cc

1-8. same was Table 59; 9. of normal goat (control); 10. anti-O serum No 2; 11. anti-O intestine No 3; 12. O-serum; 13. H-serum; 14. O-intestine; 15. H-intestine

Therefore, the aggravating effect of the injection of cytotoxic sera on the course of radiation sickness is constantly observed in the animals, regardless of whether the antiserum is obtained as the result of immunization with tissues of intact or tissues of irradiated animals.

2. The Favorable Effect of Adsorbed Sera

In noninfectious immunology extensive use is made of the adsorption of immune sera according to the Castellani principle. For example, in obtaining organ-specific sera the animals are immunized with tissues of the corresponding organ, and the serum obtained is adsorbed by the tissues of other organs. When adsorption from serum is carried out antibodies are eliminated against antigens common to all tissues: species, group, et cetera. By this technique sera with narrow specificity, which react selectively only with antigens of the corresponding organ, can be obtained (P. N. Kosyakov, 1954). By a similar technique sera with narrow specificity against cancer can be obtained. In the latter cases the animals are immunized with pathological tissues and the sera obtained are adsorbed by normal tissues, and antibodies against the pathological antigens remain in it (G. I. Abelev and others, 1960; L. A. Zil'ber, 1958; L. N. Mayskiy, 1955).

We used this principle also, counting on the fact that adsorption of sera against tissues of irradiated animals by tissues of normal animals would eliminate their cytotoxic activity and would increase the relative content of antibodies against "radiation antigens." This would make it possible to use them for experimental radiation sickness with the aim of neutralizing tissue antigens circulating in the blood and possessing toxicity, for example, intestinal mucosa. Antigens of intestinal mucosal tissue are of interest in connection with the marked and early changes occurring in it after irradiation, which have been shown above; in connection with the considerable increase in toxicity of its cellular components; with the demonstration of the state of sensitization in irradiated animals specifically with respect to this tissue as well as in conjunction with the high degree of absorptive power of the intestine, which contributes to the penetration of tissue antigens into the blood stream.

These considerations were responsible for the choice of tissue for the purpose of obtaining antisera in our experiments. We rightfully suppose that among the tissue antigens which can be of pathogenetic significance autoantigens from the intestinal mucosa are of

the greatest importance. A. S. Shevelev (1960) performed similar experiments with immune sera against the livers of irradiated mice. In a number of experiments a pronounced therapeutic effect was observed.

We made a study of a large number of sera obtained as the result of immunization of rabbits with rat intestinal mucosal tissue. The immunization plan has been described in the previous section. The sera were adsorbed by the P. N. Kosyakov method with tissue of normal formalized rat liver. For this purpose, immediately after decapitation, the livers were extracted from rats, ground up in a high-speed homogenizer with physiological saline solution, 1:10, and then poured over with an equal volume of 10-percent neutral formalin, so that the final concentration of it was equal to five percent. This tissue was used in the experiment no sooner than two weeks after being poured over with formalin. Before use, the tissue was washed repeatedly in physiological saline solution in a centrifuge at a rate of 3500 revolutions per minute. Before adsorption the serum was inactivated at 56° C for 30 minutes. Three cc of physiological saline and one gram of washed-out liver tissue were used per cc of serum. The mixture was incubated at 3° C for an hour then was centrifuged at 6000 revolutions per minute. Before and after adsorption the serum titers against extracts of intestinal mucosae of irradiated and normal rats as well as against extracts of the livers of normal rats were determined by the complement-fixation test. These three antigens characterized the serum in its three main indices: affinity for intestinal mucosa of irradiated animals, that is, specificity; affinity for normal intestinal antigens; and the presence of nonspecific antibodies to other tissues, particularly liver. It should be noted that although the immunization was untypical we obtained antisera which were far from being untypical.

In Table 61 data of several experiments are presented, on the basis of which it may be said that during immunization with intestinal mucosa from irradiated rats the sera have a somewhat greater titer with respect to irradiated tissue. In the case of immunization with normal tissues the opposite is always observed: the titers were considerably higher with respect to normal mucosa. This was constantly observed. In doses which contained the same amount of protein, the antigen prepared from the mucosa of irradiated rats reacted two-four times less strongly with the serum against normal

Table 61

Titers of Some Antisera Before and After Adsorption

| Сыв. с-тка | № сыворо-тки | До адсорбции | | | После адсорбции | | |
|---|--------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| | | 5 титр О- инт. | 6 титр Н- инт. | 7 титр Н- печ. | 5 титр О- инт. | 6 титр Н- инт. | 7 титр Н- печ. |
| Против кишечника крыс, облученных через двое суток в дозе 2000 р 8 | 40 | 1:320 | 1:80 | 1:40 | 1:64 | 1:16 | 1:4 |
| | 41 | 1:320 | 1:160 | 1:40 | 1:32 | 1:16 | 1:1 |
| | 618 | 1:160 | 1:160 | 1:40 | 1:16 | 1:16 | 1:8 |
| | 647 | 1:160 | 1:80 | 1:40 | 1:16 | 1:16 | 1:4 |
| | 636 | 1:320 | 1:320 | 1:80 | 1:32 | 1:32 | 1:8 |
| | 658 | 1:160 | 1:160 | 1:40 | 1:8 | 1:8 | 1:2 |
| | 677 | 1:160 | 1:160 | 1:80 | 1:32 | 1:32 | 1:4 |
| | 633 | 1:160 | 1:160 | 1:80 | 1:32 | 1:16 | 1:4 |
| | 679 | 1:160 | 1:80 | 1:20 | 1:16 | 1:16 | 1:2 |
| | 670 | 1:80 | 1:40 | 1:10 | 1:16 | 1:16 | 1:2 |
| | 999 | 1:160 | 1:160 | 1:80 | 1:32 | 1:16 | 1:4 |
| | 983 | 1:640 | 1:320 | 1:40 | 1:128 | 1:64 | 1:2 |
| | 989 | 1:320 | 1:320 | 1:10 | 1:64 | 1:64 | 1:2 |
| | 955 | 1:320 | 1:160 | 1:40 | 1:64 | 1:32 | 1:1 |
| | 984 | 1:160 | 1:80 | 1:80 | 1:16 | 1:16 | 1:2 |
| | 251 | 1:320 | 1:80 | 1:40 | 1:64 | 1:64 | 1:2 |
| | 1773 | 1:80 | 1:80 | 1:10 | 1:16 | 1:16 | 1:1 |
| | 2306 | 1:640 | 1:320 | 1:40 | 1:64 | 1:64 | 1:4 |
| 9 Против кишечника нор- мальных крыс | 1231 | 1:40 | 1:160 | 1:10 | 1:4 | 1:16 | 1:2 |
| | 1210 | 1:80 | 1:160 | 1:10 | 1:8 | 1:16 | 1:2 |
| | 1280 | 1:20 | 1:80 | 1:5 | 1:4 | 1:16 | 1:1 |
| | 1218 | 1:40 | 1:160 | 1:10 | 1:16 | 1:32 | 1:1 |
| | 1277 | 1:160 | 1:320 | 1:20 | 1:32 | 1:32 | 1:1 |
| | 1281 | 1:40 | 1:320 | 1:20 | 1:8 | 1:32 | 1:2 |
| | 1284 | 1:20 | 1:160 | 1:10 | — | — | — |
| | 1287 | 1:80 | 1:320 | 1:40 | — | — | — |

1. serum; 2. number of serum; 3. before adsorption; 4. after adsorption; 5. O-intestine; 6. H-intestine; 7. H-liver; 8. against intestines of rats irradiated after two days with a dose of 2000 r; 9. against the intestines of normal rats.

mucosa in the complement-fixation test. Therefore, the tissue from irradiated animals possesses less antigenicity. Despite this, in performing the complement-fixation test with serum against the intestine of irradiated rats this antigen reacts in titers equal to the normal, and in half the cases the sera show a slightly higher titer with respect to it. This higher titer is essentially more than one dilution. For each experiment of testing sera on rats we immunized a group of 7-10 rabbits. The complement-fixation tests were performed in a single stage, simultaneously with all sera. In view of the fact that antigens of the same batches were used for simultaneous tests for all sera, we were able to judge the affinity of various sera for the antigens used in the reaction with a high degree of accuracy, because thereby possible variations in the titers due to the addition of different quantities of antigen to different sera were ruled out.

After checking the batch of antisera obtained by the complement-fixation test, we were able to select the sera whose characteristic indices interested us for experiments on rats. The sera with predominant titers against the intestinal mucosae of irradiated animals and the sera which did not show such predominance were tested. Their effects on the survival rates of rats irradiated with gamma-rays on an EGO-2 apparatus with a dose of 650 r ($LD_{80/30}$) differed. The

sera were administered intramuscularly three times, just as in the previous experiments: 18 hours before irradiation, 15 minutes and six hours after irradiation. This injection plan was adopted on the basis of experience of passive immunization in toxoinfections. It is well known that the effectiveness of passive immunization is greatest when antibodies enter the blood stream before the onset of intoxication. Therefore, the first injection of serum was given before irradiation. The control animals received the sera of intact rabbits. The doses of sera are indicated in the tables.

A beneficial effect of the sera on the course of radiation sickness was recorded only in those cases where they showed predominant titers with respect to the intestinal tissues of irradiated animals and little affinity for other tissues (Tables 62-64).

In Table 65 the total results of the effects of antisera against intestinal mucosa of irradiated rats are summarized. As controls, the mortality rate figures in all control groups of experiments described in this chapter were used. One experiment was performed with the aim of finding out the possibility of a favorable effect on the course of irradiation sickness by immune serum against the intestines

Table 62

The Effect of Immune Serum on the Survival of Rats Irradiated with a Dose of 650 r

| Введенная сыворотка | Первая инъекция | Вторая инъекция | Третья инъекция | Анти-сыворотки по отношению к различным тканям | Продолжительность жизни, суток | | | | | | | Полное число | Всего |
|--|-----------------|-----------------|-----------------|--|--|---|----|----|----|----|----|--------------|-------|
| | | | | | 2 | 6 | 10 | 14 | 18 | 22 | 26 | 30 | |
| Контроль (сыворотка нормального кролика) (1) | 0,5 | 1,0 | 1,0 | — | <div> <div></div> <div></div> <div></div> <div></div> <div></div> <div></div> <div></div> </div> | | | | | | | 8 | 2 |
| Анти-О кишечник, адсорбированная № 41 (10) | 0,5 | 1,0 | 1,0 | (11) О-кишечник 1 : 32 (12) Н-кишечник 1 : 16 (13) Н-печень 1 : 1 | <div> <div></div> <div></div> <div></div> <div></div> <div></div> <div></div> <div></div> </div> | | | | | | | 4 | 6 |
| Анти-О кишечник, адсорбированная № 41 (10) | 0,1 | 0,2 | 0,2 | (13) Н-печень 1 : 1 | <div> <div></div> <div></div> <div></div> <div></div> <div></div> <div></div> <div></div> </div> | | | | | | | 5 | 5 |

* dose in cc. 1. serum injected; 2. first injection; 3. second injection; 4. third injection; 5. serum titer with respect to various tissues; 6. lifespan, days; 7. died; 8. lived; 9. control (serum of normal rabbit); 10. anti-O-intestine, adsorbed No 41; 11. O-intestine; 12. H-intestine; 13. H-liver.

The Effect of Immune Serum on the Survival of White Rats Irradiated with a Dose of 650 r

dose in cc. 1-13, same as Table 62.

continuation of Table 63

| 1 Введенная сыворотка | 2 Первое введение в cc. | 3 Второе введение в cc. | 4 Третье введение в cc. | 5 Титр сыворотки по отношению к различным жизненным жидкостям | 6 Продолжительность жизни, суток | | | | | | | | 7 Поло | 8 Выжило |
|---|----------------------------|----------------------------|----------------------------|--|-------------------------------------|---|----|----|----|----|----|----|-----------|-------------|
| | | | | | 2 | 6 | 10 | 14 | 18 | 22 | 26 | 30 | | |
| 9 Анти-О кишечник, адсорбирована № 255 | 0,1 | 0,2 | 0,2 | 10 О-кишечник 1 : 64 11 Н-кишечник 1 : 32 12 Н-печень 1 : 1 | | | | | | | | | 6 | 4 |
| | | | | | | | | | | | | | | |
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| | | | | | | | | | | | | | | |
| 9 Анти-О кишечник, адсорбирована № 255 | 0,5 | 1,0 | 1,0 | | | | | | | | | | 5 | 5 |
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* dose in cc. 1-8. same as Table 62; 9. anti-O intestine, adsorbed; 10. O-intestine;
11. H-intestine; 12. H-liver.

Table 64

The Effect of Immune Serum on the Survival of Rats Irradiated with a Dose of 650 r

| 1 Введенная сыворотка | 2 Первое дозе | 3 Второе дозе | 4 Третье дозе | 5 Титр сыворотки по отношению к различным тканям | 6 Продолжительность жизни, суток | | | | | | | | 7 Поло | 8 Важно |
|--|------------------|------------------|------------------|--|-------------------------------------|---|----|----|----|----|----|----|-----------|------------|
| | | | | | 2 | 6 | 10 | 14 | 18 | 22 | 26 | 30 | | |
| 9 Нормального кролика (контроль) | 0.1 | 0.2 | 0.2 | — | | | | | | | | | 7 | 3 |
| | | | | | | | | | | | | | | |
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| | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | |
| 10 Анти-О кишечник, аморбиролаз № 638 | 0.1 | 0.2 | 0.2 | 11 О-кишечник 1:32 12 Н-кишечник 1:16 13 Н-печень 1:4 | | | | | | | | | 4 | 6 |
| | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | |
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| | | | | | | | | | | | | | | |

* dose in cc. 1-13. same as Table 62.

of irradiated rats when it was injected after irradiation of the animals.

In all the previous experiments serum had been injected initially 18 hours before irradiation. In this experiment (Table 66) it was injected for the first time (0.5 cc) after 15 minutes; the second time (0.15 cc), after six hours; the third time (0.25 cc), 24 hours after irradiation with the following results: five out of 20 control animals survived; 11 out of 20 treated animals. Despite the fact that the control animals always received the sera of intact rabbits in the appropriate quantities, we considered it necessary to perform an experiment for finding out the effect of this normal rabbit serum injection plan on the survival rate of irradiated rats. No effect was found (Table 67). This is in agreement with data in the literature, which assert that a protective effect from the injection of heterologous sera can be recorded only when they are injected 10-12 days before irradiation. When they are injected immediately after irradiation the sera do not exert any appreciable influence or even increase the mortality rate (Graham, 1949; V. G. Kulikova and others, 1959).

Table 65

The Beneficial Effect of Adsorbed Antisera Against the Intestines of Irradiated Rats on the Survival of Rats after Irradiation with a Dose of 650 r

| Группа ① | Число животных ② | Умерло ③ | Выжило ④ | Выживаемость % ⑤ | Критерий достоверности разницы ⑥ | |
|---------------|------------------|----------|----------|------------------|----------------------------------|-------|
| | | | | | χ^2 | P |
| Контрольные ⑦ | 134 | 111 | 23 | 18 | 22,56 | 0,001 |
| Опытные ⑧ | 80 | 29 | 31 | 51 | | |

1. group; 2. number of animals; 3. died; 4. survived; 5. survival, percent; 6. criterion of the significance of the difference; 7. control; 8. experimental.

Table 66

The Effect of Immune Serum on the Survival of Rats Irradiated with a Dose of 650 r. Serum Injected Only after Irradiation

| 1 Введенная сыворотка | 2 Первая доза | 3 Вторая доза | 4 Третья доза | 5 Тип сыворотки по отношению к различным трансам | 6 Продолжительность жизни, сутки | | | | | | | | 7 План | 8 Результат |
|-------------------------------------|------------------|------------------|------------------|---|-------------------------------------|---|----|----|----|----|----|----|-----------|----------------|
| | | | | | 2 | 6 | 10 | 17 | 18 | 22 | 26 | 30 | | |
| 9 Нормального кролика (контроль) | 0.5 | 0.25 | 0.25 | — | — | — | — | — | — | — | — | — | 15 | 5 |
| | | | | | — | — | — | — | — | — | — | — | | |
| | | | | | — | — | — | — | — | — | — | — | | |
| | | | | | — | — | — | — | — | — | — | — | | |
| | | | | | — | — | — | — | — | — | — | — | | |
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| | | | | | — | — | — | — | — | — | — | — | | |
| | | | | | — | — | — | — | — | — | — | — | | |
| | | | | | — | — | — | — | — | — | — | — | | |
| | | | | | — | — | — | — | — | — | — | — | | |

1-9. same as in Table 62.

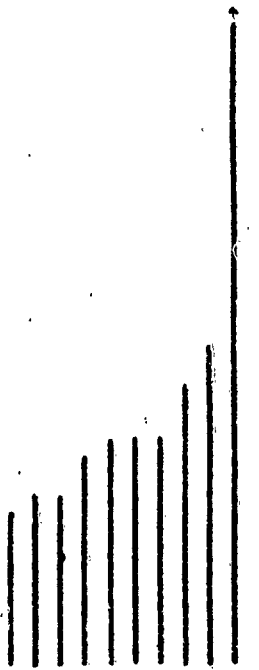
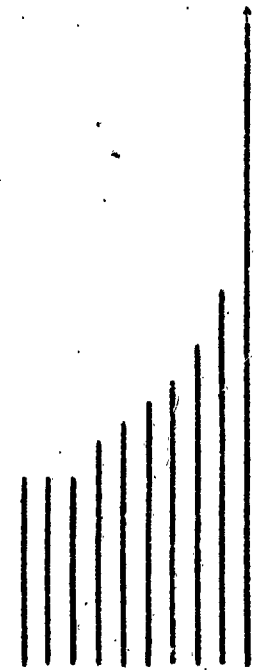
continuation of Table 66

| 1 | 2 | 3 | 4 | 5 | 6 | | | | | | | | | | 7 | 8 |
|--|-----------------|----------------|-----------------|--|---|--|--|--|--|--|--|--|--|--|-------|------|
| Воспаление серозной оболочки | Торона свечи | Второе курс | Торона свечи | Торона свечи по строению клетки и раз- личиям тканей | | | | | | | | | | | Итого | 9 11 |
| (10) Анти-О кишечник защитная № 250 | 0,5 | 0,25 | 0,25 | (11) Кишечник 1:32 (12) Кишечник 1:16 (13) Носовый 1:1 | | | | | | | | | | | | |
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* dose in cc. 10. anti-O-intestine, adsorbed; 11. O-intestine; 12. H-intestine; 13. H-liver;

Table 67

Results of Experiment of Injecting Sera of Intact Rabbits into Rats

| Введенная сыворотка | Первая доза, мл | Вторая доза, мл | Третья доза, мл | Доза, мг | Продолжительность жизни, сутки | | | | | | | Всего | Средняя продолжительность жизни, сутки |
|---|-----------------|-----------------|-----------------|----------|--|---|----|----|----|----|----|-------|--|
| | | | | | 2 | 6 | 10 | 14 | 18 | 22 | 26 | 30 | |
| 10 Контроль (без сыворотки) | — | — | — | 650 |  | | | | | | | 9 | 10.4 |
| | | | | | | | | | | | | | |
| 11 Сыворотка нормального кролика (адсорбированная) | 0.5 | 1.0 | 1.0 | 650 |  | | | | | | | 9 | 11.0 |
| | | | | | | | | | | | | | |

1-8. same as previous tables; 9. average lifespan, days; 10. control (without serum); 11. serum of normal rabbit (adsorbed)

continuation of Table 67

| 1 | 2 | 3 | 4 | 5 | 6 Продолжительность жизни, сутки | | | | | | | 7 | 8 | 9 | |
|--|--|-----|-----|-----|----------------------------------|---|----|----|----|----|----|---|----|---|-----|
| | | | | | 2 | 5 | 10 | 11 | 18 | 22 | 26 | | | | 30 |
| Выведена окраска | Сыворотка коровья (аксорбин-ролани) 10 | 0.1 | 0.2 | 0.2 | 650 | | | | | | | | 10 | 0 | 3.3 |
| | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | |
| Контроль (без сыворотки) 10 | | — | — | — | 800 | | | | | | | | 7 | 0 | 8.1 |
| | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | |
| Сыворотка коровья (аксорбин-ролани) 10 | 0.5 | 1.0 | 1.0 | 1.0 | 800 | | | | | | | | 7 | 0 | 6.7 |
| | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | |

* dose in cc.

Table 68

The Effect of Immune Serum on the Survival of Rats Irradiated with a Dose of 650 r

| 1 Вещество сыворотка | 2 Первое днем | 3 Второе днем | 4 Третье днем | 5 Титр сыворотки по отношению к разведенной жидкости титр | 6 Продолжительность жизни, суток | | | | | | | | | | 7 Пало | 8 Выжил |
|---|---------------------|---------------------|---------------------|---|-------------------------------------|---|----|----|----|----|----|----|--|---|-----------|------------|
| | | | | | 2 | 6 | 10 | 14 | 18 | 22 | 26 | 30 | | | | |
| 9 Нормального кролика (контроль) | 0.1 | 0.2 | 0.2 | | | | | | | | | | | 9 | 1 | |
| 10 Анти-О кишечник аксорбирована № 989 | 0.1 | 0.2 | 0.2 | 11 О-кишечник 1:64 12 Н-кишечник 1:64 13 Н-печень 1:2 | | | | | | | | | | 9 | 1 | |

* dose in cc. 1-13. same as in Table 62.

We also performed a number of experiments for the determination of antibodies against intestinal microbes in our sera, since they were prepared by immunization of nonsterile material, intestinal mucosa. It was determined that the antibody titers against the colon bacillus do not exceed the titers of normal antibodies found in the sera of intact rabbits. As an example we are giving the agglutinin titers against the colon bacillus in the sera of some rabbits:

| ① № кролика | ② До иммунизации | ③ После иммунизации |
|-------------|------------------|---------------------|
| 290 | 1/32 | 1/32 |
| 251 | 1/32 | 1/64 |
| 2312 | 1/64 | 1/64 |
| 2324 | 1/16 | 1/32 |
| 2340 | 1/32 | 1/32 |

1. No of rabbits; 2. before immunization; 3. after immunization

In the experiments of V. F. Sosova the normal antibody titers in intact rabbits were equal to 1/100. Therefore, the favorable effect of tissue antisera could not in any way be connected with antibodies against the intestinal microbes. This is also indicated by experiments showing the absence of such an effect in sera without predominant affinity for irradiated tissues. In Tables 68 and 69 the results of testing of such sera are shown.

From the Tables it is seen that the adsorbed antisera obtained as the result of immunizing rabbits with the intestines of irradiated rats without predominant affinity for the tissues of irradiated animals do not exert any beneficial effect on the survival rates of rats irradiated with a dose of 650 r. It should be noted that these experiments were performed simultaneously with those described previously (see the general controls in Tables 63, 64, 68 and 69), and their results cannot be explained by chance variations in the mortality rates in various experiments. The control and experimental rats were irradiated simultaneously in the same chamber.

Therefore, the parenteral injection of antibodies against tissue antigens altered under the influence of irradiation of the intestinal mucosa exerts a beneficial effect on the course of radiation sickness.

Table 69

The Effect of Immune Serum on the Survival of Rats Irradiated with a Dose of 650 r

| ① Введенная сыворотка | ② Первое дозирование | ③ Второе дозирование | ④ Третье дозирование | ⑤ Титр сыворотки по отношению к разлитым телятам | ⑥ Продолжительность жизни, суток | | | | | | | | ⑦ Гибель | ⑧ Выжившие |
|-------------------------------------|-------------------------|-------------------------|-------------------------|---|----------------------------------|---|----|----|----|----|----|----|-------------|---------------|
| | | | | | 2 | 6 | 10 | 14 | 18 | 22 | 26 | 30 | | |
| ⑨ Нормального кролика (контроль) | 0.1 | 0.2 | 0.5 | — | — | — | — | — | — | — | — | — | 7 | 3 |
| | | | | | — | — | — | — | — | — | — | — | | |
| | | | | | — | — | — | — | — | — | — | — | | |
| | | | | | — | — | — | — | — | — | — | — | | |
| | | | | | — | — | — | — | — | — | — | — | | |
| | | | | | — | — | — | — | — | — | — | — | | |
| | | | | | — | — | — | — | — | — | — | — | | |
| ⑨ Нормального кролика (контроль) | 0.5 | 1.0 | 1.0 | — | — | — | — | — | — | — | — | — | 7 | 3 |
| | | | | | — | — | — | — | — | — | — | — | | |
| | | | | | — | — | — | — | — | — | — | — | | |
| | | | | | — | — | — | — | — | — | — | — | | |
| | | | | | — | — | — | — | — | — | — | — | | |
| | | | | | — | — | — | — | — | — | — | — | | |
| | | | | | — | — | — | — | — | — | — | — | | |

1-9. same as Table 62.

continuation of Table 69

| 1 Введенная сыворотка | 2 Доза, мл | 3 Доза, мл | 4 Доза, мл | 5 Титр сыворотки по отношению к различным тканям | 6 Продолжительность жизни, суток | | | | | | | | 7 Пол | 8 | 9 |
|--|---------------|---------------|---------------|---|----------------------------------|-------|-------|-------|-------|-------|-------|-------|----------|-------|-------|
| | | | | | 2 | 6 | 10 | 14 | 18 | 22 | 26 | 30 | | | |
| 10 Анти-О-кишечник, адсорбирована № 670 | 0.1 | 0.2 | 0.2 | 11 О-кишечник 1 : 32 12 Н-кишечник 1 : 32 13 Н-печень 1 : 8 | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ |
| | | | | | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ |
| | | | | | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ |
| | | | | | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ |
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| | | | | | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ |
| 10 Анти-О-кишечник, адсорбирована № 670 | 0.5 | 1.0 | 1.0 | 11 О-кишечник 1 : 16 12 Н-кишечник 1 : 16 13 Н-печень 1 : 2 | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ |
| | | | | | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ |
| | | | | | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ |
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| | | | | | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ |

* dose in cc. 10. anti-O-intestine, adsorbed; 11. O-intestine; 12. H-intestine; 13. H-liver.

Immune sera containing a relatively large quantity of such antibodies can be obtained by means of immunization of rabbits with an extract of the intestinal mucosa of irradiated rats with subsequent adsorption by the liver tissues of intact rats. Adsorption is necessary for eliminating the general cytotoxic effect of antiserum. The injection of antibodies capable of combining with normal intestinal mucosal antibodies to the same degree does not exert the same effect. The favorable effect of neutralization of autoantigens from the intestinal mucosa in the case of radiation injury indicates their definite part in the pathogenesis of radiation sickness.

The results of experiments described in this chapter are similar to those which have been obtained in the field of studying the pathogenesis and treatment of burn sickness. Through the work of N. A. Fedorov and S. V. Skurkovich (1955) and then D. M. Grozdova and coauthors (1955) the circulation of autoantigens in the blood of burned organisms was shown. Convalescent sera or sera of animals immunized with antigens from the burned skin exert a favorable effect on the course of burn sickness. These sera have found clinical application.

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Conclusion

In conclusion it is expedient to present a brief summary of the material which has been accumulated, to formulate the main generalizations, and express certain ideas about each section of the immunology of acute radiation injury.

1. Antimicrobial Immunity in Radiation Injury

The most demonstrative illustration of the injurious effect of ionizing radiation on immunity are observations of increased sensitivity of irradiated animals to the pathogens of infectious diseases. These rules and regulations were confirmed in experiments with scores of different pathogens of bacterial, virus, rickettsial and fungous diseases; they were shown also with respect to conditionally pathogenic microorganisms. Exceptions to this rule are the pathogens of diseases which are not characteristic of the given species of animal. Species resistance is maintained after irradiation, although sensitivity to nonspecific intoxication is increased after the injection of large quantities of a microbial mass. It should be noted that there is an opposite conclusion in the literature with respect to the resistance of species immunity to the effect of radiation. O. P. Peterson and M. A. Kozlova believe that congenital resistance to some viruses can be eliminated by means of irradiating the animals. However, the proof presented by these authors gives rise to objections and can be evaluated differently (see Chapter 2, Section 6).

Study of the times of increased sensitivity to infection showed that they are not the same and depend on the dose of radiation, species of animal and nature of the infectious process. According to our data, for example, normalization of the natural resistance of white mice to the pathogen of gas gangrene occurs two weeks after irradiation. Under similar conditions normalization with respect to the pathogen of icterohemorrhagic leptospirosis does not occur for 10 weeks.

The answer to the question of how rapidly increased sensitivity to infection occurs after irradiation was given differently for a long time, because in some experiments increased sensitivity was recorded to infection produced immediately after irradiation; in others,

after several days. Comparison of data on different infectious diseases as well as the modelling, for different periods of time, of the course of the same infectious disease (gas gangrene) permitted us to conclude that the duration of the infectious process was of determinative significance for the results of the experiments. Testing of the sensitivity to infection with a pathogen the outcome of reaction with which is decided quickly, in one-two days, shows normal resistance of animals during the first few days after irradiation. Increased sensitivity occurs after several days (after three, on the average). If the infectious process is characterized by a progressive or long course, the increased sensitivity of the body to the pathogen of this infection is demonstrable when the infection is produced simultaneously with irradiation, by virtue of the fact that such an infectious process includes the period of increased sensitivity.

Increase in the sensitivity to bacteria is associated with quantitative and qualitative changes in the normal body microflora of animals after irradiation. The intestinal flora has been studied in the greatest detail. Our own studies, made dynamically during the development of acute radiation sickness in rats, as well as some data from the literature permit us to note an increase in the total number of microbes in the intestine, a change in the interrelationship of various representatives, the appearance of a large number of bacteria possessing hemolytic, proteolytic, indole- and hydrogen-sulfide-forming properties as well as the appearance of a greater than normal number of strains with altered properties, for example, antibody-resistant strains and strains with more pronounced pathogenicity. Whether all this is evidence of variation in the bacteria because of their living in an altered medium, selection of mutants or the result of colonization of the intestine with new representatives is a question which has not been studied at present. However, the developing dysbacteriosis undoubtedly plays a pathogenic role, because effects directed against prevention of these changes exert a beneficial influence on the course of radiation sickness. This is natural, since the rapidly developing increase in the permeability of biological barriers provides for the penetration of large numbers of microbes into the mesenteric lymph nodes and then into the blood as early as two days after irradiation. However, it should not be supposed that the increased permeability of tissues is the only and main cause of development of the constant bacteriemia, which before death of the animal is of a septic nature. For a long

time the mechanisms of clearance of the blood of foreign substances compensate for this pathological phenomenon. In this book three main mechanisms of purification of the blood are analyzed: the phagocytic activity of cells of the reticulo-endothelial system, the adsorption properties and permeability of tissues. A comparison of the dynamics of the change in these factors makes it possible to draw the following picture of the effect of radiation on the processes of elimination of foreign substances which have penetrated in the blood.

The increased tissue permeability developing quickly after irradiation contributes to the penetration of foreign substances, including bacteria, into the blood stream. However, the increased tissue permeability simultaneously contributes to accelerating the passage of the substances from the blood into the tissues. The increased adsorptive activity of many tissues developing rapidly after irradiation also, contributes even more to the latter. A combination of these two processes in a number of cases clears the blood of foreign antigens even more effectively than normally, despite the inhibition of the phagocytic power of the cells of the macrophagic system. Authors who give insufficient consideration to these factors come to the incorrect conclusion that the phagocytic power of the reticulo-endothelial system is resistant to the effect of radiation and that this power is maintained at a high level for many days after irradiation. Such conclusions are usually drawn on the basis of experiments of intravenous injection of different colloidal particles and other substances with subsequent determination of the blood clearance rate. Normal figures under these conditions do not indicate the well-being of immune mechanisms. Direct determination of the phagocytic power of reticulo-endothelial cells has shown a rapidly occurring inhibition. Increased adsorption under these conditions cannot be a factor in resistance, because phagocytosis of adsorbed bacteria is suppressed. Not only the reduction in the number of macro- and microphagocytes and inhibition of their phagocytic activity but also impairment of the power of intracellular digestion are typical. Experiments on the injection of living bacteria have shown that the engulfment of bacteria by the tissues from the blood as the result of adsorption and phagocytosis does not prevent their multiplication. Here, mention should be made of inhibition of the detoxifying power of tissues and cells of irradiated organism (the

studies of P. N. Kiselev's laboratory). It is associated with an inhibition of nonspecific bactericidal systems of the body -- properdin, lysozyme, bactericidal substances of a number of tissues as well as the bactericidal power of the skin. The relatively higher resistance of complement to the effect of radiation is very interesting.

Study of the effect of ionizing radiation on antibody production has made it possible to establish a number of the main rules and regulations and to show that these rules and regulations are general for immunization with "dead" antigens and for the course of an infectious process.

1. Irradiation performed after immunization either has no effect on antibody production or retards it somewhat.

2. Irradiation of animals in lethal or sublethal doses of radiation, performed before immunization, inhibits antibody production.

3. The inhibitory effect is, for the most part, directly proportional to the dose of radiation and depends on the time of injection of the antigen. The maximum inhibition is observed when immunization is performed one-two days after irradiation. Thereby, it is customarily considered that an absolutely complete suppression of antibody production is possible.

The material presented make it possible for us to state that this is not so, that one of the basic mechanisms of the inhibitory effect of radiation is a marked prolongation of the inductive phase of antibody formation. In connection with this, another rule can be formulated: no matter how severely antibody production is depressed, this process is not completely suppressed. However, prolongation of the inductive phase can be so great (to two-three weeks) that antibodies are not found in the blood because of the early deaths of the animals.

It should also be emphasized that repeated or multiple injections of antigen after irradiation of the animals provide for more active antibody production by comparison with the production of immune globulins are a single immunization. The observation of recent years and the conclusion that the degree of inhibition of production of various types of immune globulins is different are very important. Specifically, it has been shown that the production of incomplete antibodies is inhibited less than the production of other antibodies in radiation sickness.

In connection with the marked depressive effect of radiation on the basic immunity mechanisms, studies directed at a search for

methods of restoring the impaired functions and the most effective means of active and passive immunization in radiation sickness assume great practical importance. The control of increased tissue permeability, measures directed at raising the properdin level in the blood, blood transfusion and transfusion of leukocyte masses, transplantation of hemopoietic tissue -- these are the main trends in the studies for restoration of the mechanisms of natural immunity in radiation sickness. Unfortunately, the development of these trends is still far from completion. The method of transplantation of hemopoietic tissues is most promising.

Experiments on artificial immunization under conditions of radiation injury to the body have given results which are different for antitoxic and antibacterial immunity. It has been determined that irradiation of immunized animals considerably depresses their degree of resistance when infected with living pathogens during the first few days after irradiation and causes complete suppression of acquired immunity when they are infected during the period of the developed clinical picture of acute radiation sickness. However, active antimicrobial immunity, even though markedly depressed, assures a somewhat higher resistance of irradiated animals to the corresponding pathogen by comparison with irradiated nonimmunized animals. As far as active antitoxic immunity is concerned, the strength of it, created before irradiation, is to a large degree maintained after irradiation. The effectiveness of active immunization of irradiated animals depends on the time of injection of the antigen. Vaccination in the first two or three days after irradiation does not increase the resistance which has been reduced as the result of irradiation. Later immunization increases the resistance. This rule is a general one for both antibacterial and antitoxic immunity. Thereby, it should be kept in mind that during the acute period of radiation sickness animals show increased sensitivity to vaccination. Immunization aggravates the course of radiation sickness and increases the mortality rate. Conversely, the injection of microbial antigens several days before irradiation exerts a favorable effect on the course of radiation sickness. At the present time, the latter phenomenon is being studied in detail by N. N. Klemparskaya.

A difference between the effectiveness of antitoxic and antibacterial immunity is demonstrated through the passive immunization of irradiated animals. The injection of prepared antibodies does not protect the animals against subsequent infection with living

microorganisms but proves to be very effective against injection of toxins. While the sensitivity of immunized animals is increased by hundreds of times with respect to living pathogens under the influence of irradiation, two or three doses of antitoxic sera are sufficient to create the normal level of antitoxic immunity. The toxin-neutralizing power of antibodies in the irradiated organism is maintained, and this assures effectiveness of antitoxic immunity, although less than normal; however, this is absolutely inadequate for maintaining antibacterial immunity, which is largely cellular.

The practical conclusions which may be drawn from the principles presented are evident.

2. Infectious Processes in Radiation Injury

As the result of depression of anti-infectious immunity in radiation sickness infectious complications appear. Study of them is very important and interesting for the following reasons. First of all, one of the components of the pathogenesis of radiation sickness is the occurrence of endogenous infection at certain stages of the course of the main pathological process. Secondly, an infectious disease which occurs in an organism injured by penetrating radiation has a unique course. Thirdly, the problem of preventing and eliminating infectious complications after irradiation is part of the problem of treating radiation sickness.

The sources of endogenous infection developing in radiation sickness as the result of marked depression of anti-infectious immunity are the natural body microflora: the microbe-inhabitants of the intestine, respiratory tract, and others. In the presence of local infectious processes they can also be sources of bacteriemia. Penetration of microbes into the internal medium of the organism begins one-two days after irradiation and lasts for three-four weeks. When animals are irradiated with minimum absolutely lethal doses of radiation the following periods of development of autoinfection can be distinguished: the period of sterility (first day), the period of seeding of regional lymph nodes (second-third day), the bacteriemic period or the period of relative compensation of the reticulo-endothelial system (third-seventh day), and the period of decompensation or septic period. With various doses of radiation and in different animals these periods do not occur at the same times or do not occur at all. With low nonlethal doses the period of decompen-

sation does not develop; the bacteriemic period occurs later.

Endogenous infection develops constantly in acute radiation sickness but is not an absolutely necessary link in the pathogenetic chain of injury. It constitutes a complication of the main pathological process, aggravates it, and in a number of cases serves as the direct cause of death. In connection with this, antibiotic therapy, which is expediently given with the use of certain principles as guides, is obligatory and very successful in radiation sickness. These principles should provide for the early and prolonged use of broad-spectrum antibiotics, best in courses with alternation of preparations. It is advisable to create bacteriostatic concentrations of the antibiotics in places of the natural habitats of commensal microbes, to give the therapy against the background of administration of antihistamines, antihemorrhagic preparations and vitamins, as well as to exercise control over the state of immunological reactivity of the body. Certainly, these principles do not solve the problem of controlling autoinfection in radiation sickness. The solution of it lies in the field of measures directed at restoration of the impaired immunity after irradiation. With the observance of these principles in experiments on white rats the survival of 50 percent of the animals can be achieved with 17 percent survival in the controls.

A serious danger for the immunologically "disarmed" organism injured by ionizing radiation is constituted by exogenous infections. The characteristics of its reaction with the pathogens of infectious diseases are manifested not only in increased sensitivity to infections but also in the distinctive nature of the course of the infectious process. These distinctive features touch on all its main aspects. The predominance of the necrotic component and hemorrhages with inhibition of development and sometimes complete absence of the cellular component of inflammation are typical of inflammatory foci. In foci of inflammation as well as in the blood and tissues pathogens accumulate in numbers which are tens and hundreds of times greater than normal in the case of disseminated infections. Dissemination of the infection occurs sooner and can develop in focal infections, for which dissemination is not typical under normal conditions. In a number of cases the incubation period is reduced. Elimination of the pathogens from the body is markedly delayed, which may be of serious epidemiological significance. The inhibition of antibody production and the power of developing allergic reactions as well as distortion of the latter reduce and in some cases nullify the diagnostic value of serological and allergic tests. In irradiated organisms only the isolation of the culture of the specific pathogen can be considered

an absolutely reliable diagnostic sign of one disease or another, because many clinical manifestations can be distorted; temperature and leukocyte reactions may be unusual or absent. Leukocytosis typical of many infectious processes develops in irradiated animals only in the early periods after the effect of radiation, being rapidly replaced by leukopenia typical of radiation sickness.

It should be emphasized that the uniqueness of the manifestations of infections is not the result of a simple summation of the manifestations of two pathological processes; they are the result of a complex interaction of them which may be of at least two kinds. First of all, a neutral aggravation of both pathological processes. Secondly, a distinctive "quenching" of one process by the other. In different infectious diseases the latter is not conditioned by the same mechanisms. For example, reduction of parasitemia in case of infection of irradiated mice with *Plasmodium berghei* is explained by the impairment of erythropoiesis after irradiation, the reduction of young forms of erythrocytes in the blood with the inability of the plasmodia to utilize the mature forms of mouse erythrocytes. Delay in the development of tetanus intoxication under certain experimental conditions, after infection of irradiated animals, may be explained by differently directed functional changes in the central nervous system under the influence of the two pathological factors. However, everything stated does not mean that the specific nature of the infectious process disappears from irradiated animals. All the basic manifestations associated with the tropism of the pathogen, its direct biochemical activity and growth dynamics are maintained: the influenza virus affects the respiratory tract; gas gangrene occurs with the main syndrome of gas edema; in icterohemorrhagic leptospirosis a striking picture of jaundice develops, et cetera.

The fact that the activation of a latent infection is possible in radiation sickness also requires some explanation. This rule does not apply to all infections. For example, it is impossible to produce activation of a latent gas gangrene infection, because irradiation does not create the necessary condition -- the presence of necrotic tissues. An attempt to cause a recurrence of leptospirosis is also unsuccessful. Apparently, the latter is explained by the great role of antibodies in immunity in the case of leptospirosis. The presence of a high antibody level in the blood, which is not reduced by irradiation, does not permit conditions for the activation of leptospirosis infection when the pathogen is present in the kidneys.

Treatment of infectious diseases under the conditions of radiation injury is a very difficult problem which is far from being worked out yet. It may be stated that when an infectious disease occurs in an irradiated organism the therapy of it should be begun as soon as possible and should be comprehensive, directed simultaneously at all the essential pathogenetic links in the infection.

3. Noninfectious Immunology of Radiation Injury

At the present time, the existence of two kinds of autoantigens capable of leading to autoimmunization of the organism has been firmly established. Some normal tissues can be autoantigens when they enter the blood stream, where they do not come under normal conditions (brain, testicles, thyroid gland and others). Pathologically altered proteins and associated substances can also be autoantigens. After the effect of ionizing radiation on the body the real possibility for coming up against autoantigens of both kinds is created, because a rapidly developing tissue destruction is observed in combination with a marked increase in permeability of biological barriers and change in the antigenic properties of tissues. We showed the latter for various tissues and organs with the utilization of tissues of the same animal taken before and after irradiation, that is, under conditions excluding isoantigenic differences in the proteins being compared. A study was made of the antigenic characteristics of a number of whole tissues (blood, bone marrow, spleen, liver, intestinal mucosa, kidneys) as well as isolated cell microstructures -- nuclei, mitochondria, microsomes, and hyaloplasm. The antigenic properties change along two lines: the appearance of qualities not characteristic of the normal and disappearance of some of the normal antigens. For example, in the liver cell nuclei only loss of some of the antigens is observed, while in the microsomes, along with this, the appearance of an antigenic quality not characteristic of the normal is seen. The changes do not affect the species specificity; they occur chiefly with respect to organ and organoid specificity.

The redistribution of tissue proteins observed after irradiation and realized through the blood stream cannot explain changes in the antigenic properties of tissues of one organ or another. First of all, because there are data in existence which describe the acquisition of autoantigenic activity of a section of an organ (liver)

which was locally irradiated. Secondly, change in the antigenic properties occurs even after the irradiation of living isolated tissues in vitro, that is, under conditions which completely exclude any redistribution. Thirdly, this is indicated by the different directions of changes in the various structures of the same tissue and in different organs as well as the absence of new antigenic complexes in such a tissue as renal tissue. Finally, a favorable effect on the course of radiation sickness is exerted only by immune tissue antisera in which there are predominant antibody titers against tissues taken from irradiated animals with minimum titers against normal tissues.

For the same reasons, the penetration of exogenous substances into the blood and tissues cannot be considered the cause of changes in the antigens. In addition, it is impossible to tie in the phenomenon of simplification of antigenic structure of a number of tissues of irradiated animals with exogenous antigens. In connection with this, we had to explain the reason for the change in the antigenic properties of proteins and associated substances of the irradiated organism by the denaturing effect of radiation and impairment of the normal protein metabolism. This hypothesis was corroborated by special studies illustrating the distortion of protein and nucleic acid synthesis.

Summing up the change in tissues antigens, it should be emphasized that we were unable to demonstrate specific "radiation" antigens by comparing tissues taken from irradiated animals and animals with burns or inflammatory processes. Conversely, in burn sickness the appearance of a larger number of new antigenic qualities was recorded than in radiation sickness. These data certainly require further detail along the line of obtaining factual material for different doses of radiation and at various periods after irradiation. A comparison of different organs should also be made. Actually, the unique nature of the radiation injury consists of the fact that the conditions for change in the antigenic properties of proteins are observed in all tissues in connection with a whole body irradiation, whereas in a burn new antigenic substances occur only in the burned skin. The degree of heterology of the autoantigens in the latter case will certainly be greater, at least because of the grosser thermal effect. It is most logical to believe that the peculiarities in the occurrence of autoantigens in radiation sickness do not consist of the formation of some antigens

specific only for radiation injury (a dying cell can hardly synthesize something absolutely specific) but rather of the multiplicity of tissue sources of autoantigens. In other words, substances of different tissues and organs containing elements of antigenic heterology of a nonspecific nature do not enter the blood stream from some single injured tissue but rather from many and may be the cause of some pathological phenomena observed in radiation sickness.

First of all, we have in mind the significance of tissue antigens circulating in the blood as the material substrate of toxemia.

The toxicity which has been shown in the parenteral administration of some cell structures (mitochondria, microsomes) of a number of tissues and the increase in toxicity after irradiation by 5-10 times are direct evidence of this. Thereby, more pronounced toxicity of tissues obtained from irradiated animals is illustrated not only by their smaller lethal doses but also by finer indices detected by electrophysiological methods and by means of determination of the sensitivity to infection in animals which have received nonlethal doses of active tissue preparations. It is very probable that the negative results of the search for toxins in the blood of irradiated animals in the works of a number of authors are explained by the fact that these studies attempt to find some definite agent determinable by ordinary chemical or biochemical methods. Naturally, with such a manner of formulation of the study the body's own tissue proteins, showing only slight or nonspecific structural changes, remain undetected.

The second probable biologically active role of circulation of tissue antigens is associated with the possibility of immunological realization of the autoantigenic stimulus. An analysis of the nature and times of injury to antibody production in the irradiated organism as well as direct experiments on the detection of autoantibodies prove the possibility of such realization. This is also evidenced by data demonstrating the existence of increased sensitivity of irradiated animals to tissues taken from irradiated animals of the same species.

On Fig. 28 a diagram is shown illustrating the possible pathological changes in the irradiated organism associated with change and circulation of tissue antigens. After irradiation, because of a disorder of metabolic processes including the direct denaturing effect of ionizing radiation, changes in antigenic properties of a dual nature occur in proteins: loss of part of the normal antigens and the appearance of antigenic qualities not characteristic of the normal.

In addition, lethal injuries of cells can bring about the occurrence of abnormal antigenic qualities as the result of postmortem changes. Loss of some of the normal antigens, signifying a loss of certain structures, may be the cause of derangement of certain functions of cells and of an organ. This, as well as the breakdown of cells and the circulation of tissue antigens in the blood, contributes to the development of toxemia. In addition, the circulation of tissue antigens in the blood leads to an immunological rearrangement of the organism -- sensitization and antibody production. The existence of two kinds of antibodies has been shown -- against denaturing proteins and against autologous tissues. As early as at the time of appearance of antigenic heterology the altered proteins can be the cause of pathological afferent impulses. The pathological effect on chemoreceptors may be exerted subsequently also (Fig. 28), thereby providing a pathological influence through the central nervous system; this is included in the field of neuro-physiologists and pathophysiologists and has been described in a number of works (P. D. Gorizontov, A. V. Lebedinskiy, M. N. Livanov, I. A. Pigalev).

Therefore, noninfectious immunology of radiation sickness is at the present time confronted by solidly established facts illustrating all stages in the autoimmunological changes in the irradiated organism: the formation of autoantigens, circulation of them, the appearance of autoantibodies and the presence of autosensitization.

It is very difficult to speak of times at which various auto-immunological mechanisms are included in the pathogenesis of radiation sickness. Some may be of importance in the initial periods; others, during remote sequelae. Nevertheless, existing data make it possible to construct a schema for the development of autoimmunization and autosensitization in radiation sickness and to suppose that the part played by these processes in the pathogenesis of radiation sickness is a significant one. The latter has been confirmed by certain data on the therapeutic effectiveness of nonspecific desensitizing agents in radiation sickness, by a number of experimental proofs obtained by N. N. Klemparskaya as well as by our own data on the favorable effect of immune sera against tissues of irradiated animals on the course of radiation sickness. These data indicate directly that the fixation of auto-antigens by means of artificially obtained and parenterally administered

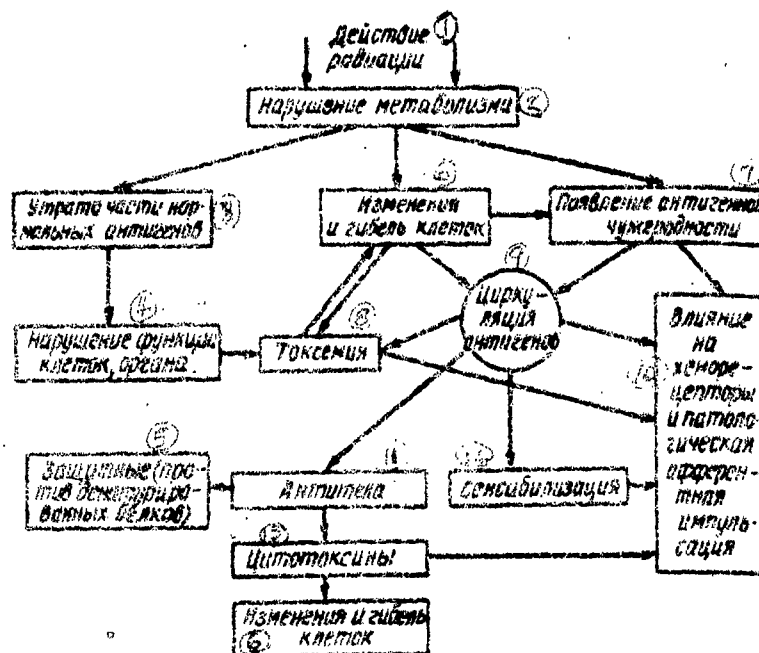


Fig. 28. Diagram Showing the Sequence of Processes Associated with the Alteration of Antigenic Properties of Tissues and the Circulation of Tissue Antigens after Irradiation. 1. radiation effect; 2. metabolic disorder; 3. loss of some of the normal antigens; 4. derangement of function of cells or organ; 5. defense (against denatured proteins); 6. changes and death of cells; 7. manifestation of antigenic heterology; 8. toxemia; 9. circulation of antigens; 10. effect on chemoreceptors and pathological afferent impulses; 11. antibodies; 12. sensitization; 13. cytotoxins.

antibodies against them mitigates the course of acute radiation sickness and reduces the animal mortality rate. In experiments on white rats we have shown that when they are injected three times with adsorbed immune sera against the intestinal mucosal tissues of irradiated rats the survival rate of these animals is 52 percent, as

against 19 percent survival among the radiation controls.

In conclusion, we should characterize the dynamics of C-reactive protein in radiation sickness and its significance. This protein appears in the blood of monkeys three hours after irradiation and reaches a maximum after 9-12 hours. In cases of a rapid course of radiation sickness, where the monkeys live a total of four-six days, the C-reactive protein does not disappear from the blood until death occurs. In the other cases, C-reactive protein disappears from the blood two or three days after irradiation. C-reactive protein always reappears two-three days before death, and in cases of recovery a second-wave of appearance of C-reactive protein documents the most severe period of radiation sickness. C-reactive protein appears in the blood of patients after x-ray therapy along with the development of the so-called radiation reaction.

Since C-reactive protein is an index of tissue destruction, the test for this protein can prove to be very useful in radiation therapy clinics for evaluating the injurious effect of radiation. The specific dynamics of its occurrence in the blood in acute radiation sickness also make it possible to study the rates and intensity of development of the pathological process and to orient the physician with respect to the critical period for the life of the patient.

As far as the pathogenetic significance of occurrence of C-reactive protein after irradiation is concerned, only some suppositions can be expressed which are different in accordance with the tastes of one investigator or another. Being a nonspecific substance for any pathological process with antigenic qualities distinguishing it from the normal body proteins, C-reactive protein may be regarded as a nonspecific autoantigen. However, its auto-antigenicity has not been studied. Its biological tissue-injuring activity after administration to healthy individuals has been proved. In connection with this, the early accumulation of large quantities of it in the blood of irradiated animals can be considered one of the factors in toxemia. The search for antibodies against C-reactive protein will throw light on the possibility of its participation in autoimmunization or autosensitization processes.

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